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**(54) UNIVERSAL ANTIBODY-MEDIATED BIOSENSOR**

UNIVERSELLER ANTIKÖRPER-VERMITTELTER BIOSENSOR

BIOCAPTEUR À MÉDIATION PAR ANTICORPS UNIVERSEL

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**Description****BACKGROUND**

5 **[0001]** There is an increasing need in the fields of food safety, health care, agricultural testing, and biodefense for affordable and highly sensitive assays that rapidly and accurately identify the presence of environmental and pathogenic agents, including toxins, antigens, bacteria, and viruses, in samples of interest. To this end, a variety of biosensor products have been commercially developed and released.

10 **[0002]** A specific example of a biosensor platform currently in use is the CANARY® biosensor technology of Path-Sensors, Inc. This platform, based on the work of Rider et al. [1], enables reliable identification of specific airborne and liquid-based pathogens. The biological backbone of the CANARY® biosensor is comprised of a genetically-engineered B cell expressing an extracellularly bound, antigen-specific antibody that can bind its cognate antigen or pathogenic agent. In this system, when an antigen-containing sample interacts with the antibody on the extracellular surface of the biosensor, an intracellular signaling cascade is activated resulting in the release of  $\text{Ca}^{2+}$  within the B cells. In the  
15 CANARY® system, the B cells express aequorin, a  $\text{Ca}^{2+}$ -sensitive photoprotein, which results in cell luminescence in the presence of elevated intracellular  $\text{Ca}^{2+}$  levels. Thus, the luminescence can be used to indicate antigen binding.

**[0003]** The CANARY® system can be used to efficiently identify a number of specific antigens, including those from bacteria, viruses, and toxins. However, expansion of the antigen test repertoire is complex and costly. Different antigen- or pathogen-specific biosensors must be constructed to recognize each and every selected antigen, which requires multiple steps including production of hybridoma cell lines, cloning of nucleic acid sequences encoding the antibodies, and expressing cloned antibodies as transmembrane proteins on the surface of a B cell line genetically engineered to luminesce upon binding of the cognate antigen (e.g., a pathogen) by the antibody.

20 **[0004]** US2012/225423A1 (25) provides methods for the detection of target particles, such as pathogens, soluble antigens, nucleic acids, toxins, chemicals, plant pathogens, blood borne pathogens, bacteria, viruses and the like. Also described is an emitter cell comprising a receptor, wherein the receptor can be an antibody or an Fc receptor, and an emitter molecule for the detection of a target particle in a sample wherein the target particle to be detected is bound by one or more receptors on the emitter cell. The document also discloses optoelectronic sensor devices for detecting a target particle in a sample, including in a plurality of samples.

25 **[0005]** Thus, the need remains for the development of a universal biosensor that can be adapted for use in multiple testing platforms across a broad range of environmental and pathogenic agents. The present invention is directed to this and other important goals.

**BRIEF SUMMARY**

35 **[0006]** Provided herein are universal antibody-mediated biosensors that can be used to detect and quantify target agents in a sample, as well as methods of using the biosensors to screen samples from a selected target agent.

**[0007]** The biosensors of the disclosure generally comprise a cell line stably expressing a novel chimeric fusion protein. The fusion protein contains an antibody-binding domain (such as the extracellular domain of an  $\text{Fc}\gamma$  receptor ( $\text{Fc}\gamma\text{R}$ )) fused to a signaling domain (such as the intracellular activation domain of immunoglobulin-alpha ( $\text{Ig}\alpha$ )). The N-terminal, extracellular antibody-binding domain has the ability to bind to the Fc region of an antibody, while the C-terminal, intracellular signaling domain has the ability to activate cellular processes, such as  $\text{Ca}^{2+}$  release. Such activation occurs when antibodies bound to the antibody-binding domain are crosslinked by their cognate antigen.

40 **[0008]** Because the antibody-binding domain of the chimeric fusion protein binds the Fc region of an antibody, the antibody that can be bound by the fusion protein is not limited by the antigenic specificity of the antibody. Thus, the chimeric fusion protein has the ability to bind any available antibody that recognizes and binds a selected target (e.g., antigen or pathogenic agent).

45 **[0009]** The biosensor of the invention provides a rapid and economical means of testing for the presence of a wide range of different target agents using the same platform, without requiring the production of separate chimeric fusion proteins for each selected target agent. This universal biosensor can be used in conjunction with commercially available antibodies as well as antibodies produced specifically to be used with the biosensor.

Fusion Proteins

55 **[0010]** In a first aspect, the description is directed to chimeric fusion proteins comprising an  $\text{Fc}\gamma$  receptor ( $\text{Fc}\gamma\text{R}$ ) antibody-binding domain, a transmembrane domain and a signaling domain. The fusion proteins have the ability to recognize and bind the Fc region of an antibody via their antibody-binding domain. The fusion proteins also have the ability to activate an intracellular signaling cascade in a cell expressing the fusion protein. In certain aspects, the intracellular signaling cascade results in the release of  $\text{Ca}^{2+}$  within the cell.

**[0011]** In certain aspects of this embodiment, the Fc $\gamma$ R antibody-binding domain is the Fc $\gamma$ RI antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3. In certain other aspects of this embodiment, the Fc $\gamma$ R antibody-binding domain is the Fc $\gamma$ RIII antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4. The sequence variants retain the antibody-binding activity of the antibody-binding domain upon which they are based.

**[0012]** In certain aspects of this embodiment, the signaling domain is the immunoglobulin alpha (Iga) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5. In certain other aspects of this embodiment, the signaling domain is the partial membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6. The sequence variants retain the signaling activity of the signaling domain upon which they are based.

**[0013]** In selected aspects, the fusion protein is the Fc $\gamma$ RI/Ig $\alpha$  fusion protein set forth in SEQ ID NO:8, the Fc $\gamma$ RIII/Ig $\alpha$  fusion protein set forth in SEQ ID NO:10, the Fc $\gamma$ RI/membrane Ig fusion protein set forth in SEQ ID NO:22, or the Fc $\gamma$ RIII/membrane Ig fusion protein set forth in SEQ ID NO:23, or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:8, 10, 22, or 23.

**[0014]** The disclosure includes polynucleotides comprising nucleotide sequences encoding each of the fusion proteins provided in the various embodiments and aspects defined herein, as well as complementary strands thereof. The disclosure also includes cloning vectors comprising the polynucleotides, and host cells comprising either the polynucleotides or the expression vectors. Such host cells may be mammalian or non-mammalian cells. The disclosure further includes methods of producing the fusion proteins defined herein, comprising culturing the host cells under conditions promoting expression of the fusion proteins encoded by the polynucleotides and expression vectors, and recovering the fusion proteins from the cells or cell cultures.

#### Biosensor Cells

**[0015]** In a second aspect, the description is directed to biosensor cells stably expressing a chimeric fusion protein, wherein the chimeric fusion protein comprises an Fc $\gamma$  receptor (Fc $\gamma$ R) antibody-binding domain, a transmembrane domain and a signaling domain. The fusion proteins have the ability to recognize and bind the Fc region of an antibody via their antibody-binding domain. The fusion proteins have the ability to activate an intracellular signaling cascade in the cell expressing the fusion protein. In certain aspects, the intracellular signaling cascade results in the release of Ca<sup>2+</sup> within the cell. The chimeric fusion protein is stably expressed on the surface of the cell as an integral membrane protein.

**[0016]** In certain aspects of this embodiment, the biosensor cell is a B cell, a T cell, a monocyte, a macrophage, a HEK293 cell, a CHO cell, P815, K562, or a Cos-1 cell, each of which stably expresses the chimeric fusion protein.

**[0017]** In certain aspects of this embodiment, the Fc $\gamma$ R antibody-binding domain is the Fc $\gamma$ RI antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3. In certain other aspects of this embodiment, the Fc $\gamma$ R antibody-binding domain is the Fc $\gamma$ RIII antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4. The sequence variants retain the antibody-binding activity of the antibody-binding domain upon which they are based.

**[0018]** In certain aspects of this embodiment, the signaling domain is the immunoglobulin alpha (Iga) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5. In certain other aspects of this embodiment, the signaling domain is the partial membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6. The sequence variants retain the signaling activity of the signaling domain upon which they are based.

#### Methods of Detecting an Agent

**[0019]** In a third aspect the description is directed to methods of detecting a target agent in a sample. The method comprises (a) contacting a sample with an antibody having binding specificity for a target agent and with a biosensor cell, and (b) assaying the biosensor cell for cellular activation, wherein the biosensor cell stably expresses a chimeric fusion protein, and wherein the chimeric fusion protein comprises an Fc $\gamma$  receptor (Fc $\gamma$ R) antibody-binding domain, a transmembrane domain and a signaling domain.

**[0020]** The fusion proteins have the ability to recognize and bind the Fc region of an antibody via their antibody-binding domain. The fusion proteins have the ability to activate an intracellular signaling cascade in the cell expressing the fusion protein. In certain aspects, the intracellular signaling cascade results in the release of Ca<sup>2+</sup> within the cell. The chimeric fusion protein is stably expressed on the surface of the cell as an integral membrane protein.

**[0021]** In certain aspects of this embodiment, the sample is an air sample, a liquid sample, a dry sample, vegetable sample, or a biological sample. In preferred aspects, when the sample is an air sample it is selected from the group

consisting of an aerosol, an atmospheric sample, a ventilator discharge, and an engine exhaust. In preferred aspects, when the sample is a liquid sample it is selected from the group consisting of a food, a drink, a water sample, a pharmaceutical formulation, and a personal care product. In preferred aspects, when the sample is a dry sample it is selected from the group consisting of food, soil, a pharmaceutical formulation, solubilized swab samples, and a personal care product. In preferred aspects, when the sample is a vegetable sample it is selected from the group consisting of leaves, fruit, nuts, seeds, flowers, and plant tissue. In preferred aspects, when the sample is a biological sample it is selected from the group consisting of blood, serum, sweat, urine, cerebrospinal fluid, mucus, semen, stool, bronchoalveolar lavage fluid, and tissue.

**[0022]** In certain aspects of this embodiment, the agent is an environmental toxin, pollutant, drug, or a biologic agent. In preferred aspects, when the agent is a biologic agent it is selected from the group consisting of a bio-warfare agent, an allergen, a parasitic antigen, a fungal antigen, a viral antigen, a bacterial antigen, a cellular antigen, and an antibody.

**[0023]** In certain aspects of this embodiment, the biosensor cell is a B cell, a T cell, a monocyte, a macrophage, a HEK293 cell, a CHO cell, P815, K562, or a Cos-1 cell, each of which stably expresses the chimeric fusion protein.

**[0024]** In certain aspects of this embodiment, the cellular activation is an increase in intracellular  $Ca^{2+}$  levels.

**[0025]** In certain aspects of this embodiment, the FcyR antibody-binding domain is the FcyRI antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3. In certain other aspects of this embodiment, the FcyR antibody-binding domain is the FcyRIII antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4. The sequence variants retain the antibody-binding activity of the antibody-binding domain upon which they are based.

**[0026]** In certain aspects of this embodiment, the signaling domain is the immunoglobulin alpha (Iga) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5. In certain other aspects of this embodiment, the signaling domain is the partial membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6. The sequence variants retain the signaling activity of the signaling domain upon which they are based.

**[0027]** The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described herein, which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that any conception and specific embodiment disclosed herein may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. The novel features which are believed to be characteristic of the invention, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that any description, figure, example, etc. is provided for the purpose of illustration and description only and is by no means intended to define the limits the invention.

## BRIEF DESCRIPTION OF DRAWINGS

### [0028]

**Figure 1.** Cartoon representation of constructs encoding fusion proteins of the disclosure. Construct A encodes the FcyRI/Iga fusion protein and construct B encodes the FcyRIII/Iga fusion protein. These fusion proteins are identical except the FcyRI/Iga fusion protein has the FcyRI antibody-binding and transmembrane domains, while the FcyRIII/Iga fusion protein has the FcyRIII antibody-binding and transmembrane domain. Construct C encodes the FcyRI/membrane Ig fusion proteins and construct D encodes the FcyRIII/membrane Ig fusion proteins. These fusion proteins are identical except the FcyRI/membrane Ig fusion protein has the FcyRI antibody-binding domain, while the FcyRIII/membrane Ig fusion protein has the FcyRIII antibody-binding domain. The membrane Ig portion of these fusion proteins comprises the hinge-CH2-CH3-transmembrane-intracellular domains from a membrane-associated antibody. Constructs E and F also encode the FcyRI/Iga and FcyRIII/Iga fusion proteins, respectively, but these constructs further encode the 2A peptide and FcR $\gamma$ -chain.

**Figure 2.** Sequence of murine FcyRI (SEQ ID NO:15). The extracellular, antibody-binding region is at N terminus; the shaded sequence is the predicted transmembrane region; the intracellular region is at the C terminus.

**Figure 3.** Sequence of the murine FcyRIII (SEQ ID NO:16). The extracellular, antibody-binding region at N terminus; the shaded sequence is the predicted transmembrane region; the intracellular region is at the C terminus.

**Figure 4.** Sequence of murine immunoglobulin alpha (Iga; CD79A; SEQ ID NO:17). The extracellular region at N terminus; the shaded sequence is the predicted transmembrane region; the intracellular region is at the C terminus.

**Figure 5.** Sequence of the FcyRI/Iga fusion protein (fusion protein A; SEQ ID NOs:7 and 8). The antibody-binding domain and transmembrane domain (shaded sequence) of FcyRI are fused to the Iga signaling domain (underlined)

in the 5' to 3' direction.

**Figure 6.** Sequence of the FcγRIII/Igα fusion protein (fusion protein B; SEQ ID NOs:9 and 10). The antibody-binding domain and transmembrane domain (shaded sequence) of FcγRIII are fused to the Igα signaling domain (underlined) in the 5' to 3' direction.

**Figure 7.** Partial sequence of a human IgG2 membrane Ig (SEQ ID NO:18). Hinge region, followed by CH<sub>2</sub> domain (underlined), CH<sub>3</sub> domain (double underlined), transmembrane domain, and intracellular domain (underlined) in the 5' to 3' direction.

**Figure 8.** Sequence of FcγRI/membrane Ig fusion protein (fusion protein C; SEQ ID NO:22). The antibody-binding domain of FcγRI is fused to the partial human IgG2 membrane Ig domain (underlined) in the 5' to 3' direction.

**Figure 9.** Sequence of FcγRIII/membrane Ig fusion protein (fusion protein D; SEQ ID NO:23). The antibody-binding domain of FcγRIII is fused to the partial human IgG2 membrane Ig domain (underlined) in the 5' to 3' direction.

**Figure 10.** Sequence of the 2A peptide (SEQ ID NO:24).

**Figure 11.** Sequence of FcRγ-chain (SEQ ID NO:25).

## DETAILED DESCRIPTION

### I. Definitions

**[0029]** Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found, for example, in Benjamin Lewin, Genes VII, published by Oxford University Press, 2000 (ISBN 019879276X); Kendrew et al. (eds.); The Encyclopedia of Molecular Biology, published by Blackwell Publishers, 1994 (ISBN 0632021829); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and other similar technical references.

**[0030]** As used herein, "a" or "an" may mean one or more. As used herein when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more. Furthermore, unless otherwise required by context, singular terms include pluralities and plural terms include the singular.

**[0031]** As used herein, "about" refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term "about" generally refers to a range of numerical values (e.g., +/- 5-10% of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). In some instances, the term "about" may include numerical values that are rounded to the nearest significant figure.

### II. The Present Invention

**[0032]** As briefly summarized above, the present description is directed to a universal antibody-mediated biosensor comprising a cell line stably expressing a novel chimeric fusion protein on its surface.

**[0033]** A first embodiment of the present invention is a method of detecting a target agent in a sample according to claim 1.

**[0034]** Further embodiments of the present invention are a biosensor cell according to claim 6 and a chimeric fusion protein according to claim 8.

**[0035]** The fusion proteins can bind antibodies without regard to their antigenic-binding specificity, and cells expressing the fusion proteins on their surface can be activated upon cross-linking of the bound antibodies by their cognate antigen. Because the fusion proteins bind to the Fc region of any antibody, they can serve as a universal pathway between extracellular signaling and intracellular activation. The biosensor can be used to detect the presence of selected antigens in a sample by contacting the sample with (i) the biosensor cells and (ii) antibodies having binding specificity for the antigen. Once added, the antibodies are bound by the chimeric fusion proteins, via binding of the Fc region of the antibody by the antibody-binding domain of the fusion proteins. Antigen recognition and binding by the antibodies leads to antibody cross-linking, which is promulgated as a signal through the fusion protein into the biosensor cell, where the intracellular signaling domain of the fusion protein triggers cellular activation. Such activation can then be assayed and, if desired, quantified. Based on the level of cellular activation, conclusions can be drawn about the presence of antigen in the sample. Very broadly speaking, when cellular activation occurs using the biosensor cells of the disclosure, the antigen is deemed to be present in the sample.

**[0036]** While the universal antibody-mediated biosensor of the description comprises a cell line stably expressing a novel chimeric fusion protein as an integral membrane protein, the individual elements of the biosensor cells include (i) an extracellular, antibody-binding domain of the fusion protein, (ii) a transmembrane domain of the fusion protein, (iii) an intracellular signaling domain of the fusion protein, and (iv) a cell line that stably expresses the fusion protein on its

surface as an integral membrane protein. These elements are discussed in the following paragraphs.

#### Antibody-binding Domain

5 **[0037]** The chimeric fusion proteins of the description comprise, at their amino termini, an extracellular, antibody-binding domain. Exemplary antibody-binding domains include, but are not limited to, the antibody-binding domain of an Fc $\gamma$  receptor (Fc $\gamma$ R), such as Fc $\gamma$ RI or Fc $\gamma$ RIII. Because different Fc $\gamma$ R subtypes vary in their affinity for different antibody isotypes (constant regions), biosensors of the description can vary based on the identity of the antibody-binding domain in the fusion protein. For example, the murine Fc $\gamma$ RI antibody-binding domain has a high-affinity for the constant regions of murine IgG2a, as well as human IgG1, IgG3 and IgG4 immunoglobulins. The antibody-binding domain of murine Fc $\gamma$ RI binds the murine IgG2a isotype with very high affinity ( $>10^8$  M $^{-1}$ ) [2]. Cross-species binding studies have demonstrated that human Fc $\gamma$ RI can bind commercially available human mAbs, with IgG1 and IgG3 binding more strongly than IgG4 [3]. The murine Fc $\gamma$ RIII antibody-binding domain has a lower affinity ( $3 \times 10^4$  to  $6 \times 10^5$  M $^{-1}$ ) for the constant regions of murine IgG1, IgG2a, IgG2b, and for human IgG1, IgG2 and IgG4 immunoglobulins [3], but can also be used in the fusion proteins of the disclosure. Between Fc $\gamma$ RI and Fc $\gamma$ RIII, all mouse and human Igs (except for murine IgG3) can bind to one of these two Fc receptors. Additionally, polyclonal antibodies can bind to these Fc $\gamma$ Rs [4].

10 **[0038]** The skilled artisan will thus understand that depending on the particular agent being assayed and the particular experimental conditions, the antibody-binding domains of different Fc $\gamma$  receptors will be preferable for different conditions. The present description is thus generally directed to novel chimeric fusion proteins comprising the antibody-binding domains of the Fc $\gamma$  receptors defined herein, as well as cell lines that stably express these fusion proteins.

15 **[0039]** In a first aspect, the antibody-binding domain of the Fc $\gamma$  receptor used in the chimeric fusion proteins includes both the antibody-binding domain and the transmembrane domain of an Fc $\gamma$  receptor. Suitable Fc $\gamma$  receptor antibody-binding/transmembrane domains include, but are not limited to, the antibody-binding/transmembrane domain of mouse Fc $\gamma$ RI set forth in SEQ ID NO:1 (where amino acids 287-319 correspond to the predicted transmembrane domain) and the antibody-binding/transmembrane domain of mouse Fc $\gamma$ RIII set forth in SEQ ID NO:2 (where amino acids 208-233 correspond to the predicted transmembrane domain).

20 **[0040]** In a second aspect, the antibody-binding domain of the Fc $\gamma$  receptor used in the chimeric fusion proteins lacks a transmembrane domain, e.g., where the transmembrane domain of the fusion protein is from an alternative source. Suitable Fc $\gamma$  receptor antibody-binding domains lacking a transmembrane domain that may be used in the chimeric fusion proteins include, but are not limited to, the antibody-binding domain of mouse Fc $\gamma$ RI set forth in SEQ ID NO:3 and the antibody-binding domain of mouse Fc $\gamma$ RIII set forth in SEQ ID NO:4.

#### Signaling Domain

25 **[0041]** The chimeric fusion proteins of the disclosure comprise, at their carboxy termini, an intracellular signaling domain. Suitable signaling domains include those known to induce cellular activation in other contexts. For example, B cells innately transduce B cell receptor (BCR) binding of an antigen through formation of a complex with the transmembrane protein CD79. CD79 is composed of two distinct chains, immunoglobulin-alpha (Ig $\alpha$ ) and immunoglobulin-beta (Ig $\beta$ ), that form the heterodimer on the surface of B cells. Ig $\alpha$  and Ig $\beta$  have an extracellular domain, a single transmembrane domain, and a cytoplasmic signaling domain. It has been demonstrated that fusion proteins with the extracellular and transmembrane regions of the CD8 protein fused to either the Ig $\alpha$  or Ig $\beta$  intracellular signaling regions have signaling capacity [5]. Other studies demonstrate that protein kinases are more potent activators of the CD8/Ig $\alpha$  fusion protein. The same study further demonstrated that Ca $^{2+}$  signaling could be observed with the CD8/Ig $\alpha$  fusion protein after CD8 cross-linking. Based on these studies, in one aspect the fusion proteins of the disclosure comprise an antibody-binding domain fused to the cytoplasmic signaling domain of Ig $\alpha$  [6].

30 **[0042]** Thus, in a first aspect, signaling domains that may be used in the chimeric fusion proteins of the invention include, but are not limited to, the signaling domain of mouse Ig $\alpha$  set forth in SEQ ID NO:5.

35 **[0043]** Since the affinity of binding between the fusion protein and antibodies can be quite variable, depending on the identity of the antibody-binding domain used in the fusion protein and the antibodies, it is important to have alternative signaling domains that can provide further nuances to the avidity of the fusion proteins for the antibodies. For example, the signaling domains may help with cross-linking and dimerization. It is thought that putting two antibody-binding domains in close proximity will increase the probability of maximal crosslinking. If antibody-binding domains are linked to a modified membrane-associated IgG molecule as the signaling domain, close proximity of two antibody-binding domains can be achieved. Thus, and in a second aspect, the signaling domain is a partial membrane Ig peptide comprising a hinge region followed by CH $_2$ , CH $_3$ , transmembrane and intracellular regions of an IgG antibody (see fusion proteins C and D in Figure 1). In a specific example, such a signaling domain is set forth in SEQ ID NO:6. As this signaling domain includes a transmembrane region, it would be used in conjunction with antibody-binding domains lacking a transmembrane domain, such as the Fc $\gamma$ RI antibody-binding domain set forth in SEQ ID NO:3 or the Fc $\gamma$ RIII antibody-binding domain

set forth in SEQ ID NO:4.

### Chimeric Fusion Proteins

**[0044]** It will be apparent that by using different combinations of antibody-binding domains and signaling domains, the affinity of the fusion proteins for a particular antibody can be adjusted and the level of cellular activation can be controlled. Specific examples of chimeric fusion proteins included in the scope of the invention include those provided in Table 1. A representation of each of the six fusion proteins is shown in Figure 1.

**Table 1**

<i><b>Fusion Protein</b></i>	<i><b>Source of Antibody-binding Domain</b></i>	<i><b>Source of Transmembrane Domain</b></i>	<i><b>Source of Signaling Domain</b></i>	<i><b>SEQ ID NO: for Nucleic Acid Sequence</b></i>	<i><b>SEQ ID NO: for Amino Acid Sequence</b></i>
Fc $\gamma$ RI/Ig $\alpha$	Fc $\gamma$ RI	Fc $\gamma$ RI	Ig $\alpha$	7	8
Fc $\gamma$ RIII/Ig $\alpha$	Fc $\gamma$ RIII	Fc $\gamma$ RIII	Ig $\alpha$	9	10
Fc $\gamma$ RI/membrane Ig	Fc $\gamma$ RI	Membrane Ig	Membrane Ig	11	12
Fc $\gamma$ RIII/membrane Ig	Fc $\gamma$ RIII	Membrane Ig	Membrane Ig	13	14

**[0045]** The invention thus includes the Fc $\gamma$ RI/Ig $\alpha$  fusion protein set forth in SEQ ID NO:8, the Fc $\gamma$ RIII/Ig $\alpha$  fusion protein set forth in SEQ ID NO:10, the Fc $\gamma$ RI/membrane Ig fusion protein set forth in SEQ ID NO:22, and the Fc $\gamma$ RIII/membrane Ig fusion protein set forth in SEQ ID NO:23.

**[0046]** Because different antibody-binding domains can be paired with different signaling domains, it should be understood that the present invention also includes fusion proteins comprising the antibody-binding domain of Fc $\gamma$ RI as set forth in SEQ ID NO:1 or 3, and fusion proteins comprising the antibody-binding domain of Fc $\gamma$ RIII as set forth in SEQ ID NO:2 or 4. Similarly, the present invention includes fusion proteins comprising the signaling domain of Ig $\alpha$  as set forth in SEQ ID NO:5, and fusion proteins comprising the signaling domain of membrane Ig as set forth in SEQ ID NO:6.

**[0047]** It will be readily understood by the skilled artisan that minor alterations can be made to the amino acid sequence of the fusion proteins of the disclosure without affecting the binding or signaling activity of the proteins. For example, minor alterations can be made to the antibody-binding domain of the fusion proteins while maintaining the binding activity of the fusion proteins. Similarly, minor alterations can be made to the signaling domain of the fusion proteins while maintaining the signaling activity of the fusion proteins. Further, minor alterations can be made to both the antibody-binding and signaling domains of the fusion proteins while maintaining the binding and signaling activity of the fusion proteins. Such minor alterations can be used to alter the affinity of the antibody-binding domain for antibodies as in some instances a particular binding affinity (e.g., low, medium or high) may be preferred. Similarly, such minor alterations can be used to alter the signaling activity of the signaling domain in a cell as in some instances a particular type or level of cellular activation (e.g., low, medium or high) may be preferred.

**[0048]** Thus, the present disclosure includes sequence variants of the fusion proteins disclosed herein having one or more amino acid insertions, deletions and/or substitutions, that also retain the binding and signaling activity of the fusion protein upon which they are based. In particular, the disclosure includes sequence variants having at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:8, 10, 22, or 23.

**[0049]** The disclosure also includes sequence variants comprising an antibody-binding domain of Fc $\gamma$ RI wherein the domain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity with SEQ ID NO:1 or 3 over the entire length of the amino acid sequence.

**[0050]** The disclosure also includes sequence variants comprising an antibody-binding domain of Fc $\gamma$ RIII wherein the domain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity with SEQ ID NO:2 or 4 over the entire length of the amino acid sequence.

**[0051]** The disclosure further includes sequence variants comprising a signaling domain of Ig $\alpha$  wherein the domain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity with SEQ ID NO:5 over the entire length of the amino acid sequence.

**[0052]** The disclosure further includes sequence variants comprising a signaling domain of membrane Ig wherein the domain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity with SEQ ID NO:6 over the entire length of the amino acid sequence.

#### 5 Polynucleotide

**[0053]** The disclosure includes polynucleotides comprising nucleotide sequences encoding each the fusion proteins provided herein, as well as complementary strands thereof. The disclosure also includes cloning vectors comprising the polynucleotides, and host cells comprising either the polynucleotides or the expression vectors. Such host cells may be mammalian or non-mammalian cells, including, but not limited to, *E. coli*, and insect cells. The disclosure further includes methods of producing the fusion proteins defined herein, comprising culturing the host cells under conditions promoting expression of the fusion proteins encoded by the polynucleotides and expression vectors, and recovering the fusion proteins from the cells or cell cultures.

#### 15 Constructs Encoding the Fusion Proteins

**[0054]** Sequences for the murine Fc $\gamma$ RI (SEQ ID NO:15) and Fc $\gamma$ RIII (SEQ ID NO:16) have been cloned and confirmed. Nucleic acid constructs encoding the chimeric fusion proteins may be generated for expression of the fusion proteins by engineering sequence encoding the antibody-binding, transmembrane, and signaling domains into an expression vector. For example, antibody-binding and transmembrane domains of the Fc $\gamma$ R receptors may be fused in frame with sequence encoding a signaling domain, for example via "SOEing" using PCR [15]. To complete the construct in the cases where the FcR- $\gamma$  chain is needed (discussed below), the C-terminus of the signaling domain and the N-terminus of the FcR- $\gamma$  chain would be attached by PCR to sequence encoding the 2A peptide. For construction of the Fc $\gamma$ R-membrane Ig constructs, restriction sites at the C-terminus of the Fc $\gamma$ R sequences may be used to link to the Ig constant regions that contain compatible restriction sites at the N-terminus.

**[0055]** Polynucleotide constructs encoding the fusion proteins of the disclosure may be transiently or stably expressed in a selected cell line. The constructs can be transfected into a selected cell line using techniques well known to the skilled artisan including, but not limited to, standard transfection kits (e.g., Fugene® or Neon™ system electroporation) or retroviral transduction methods.

**[0056]** Expression of the fusion protein on the cell surface can also be confirmed using standard techniques well known to the skilled artisan, including staining with fluorescently-labeled antibodies for either Fc $\gamma$ RI or Fc $\gamma$ RIII, and analysis using flow cytometry.

**[0057]** Suitable expression vectors include, but are not limited to, plasmids pcDNA 3.1+ or - (hygro), pcDNA 3.1 + or - (neomycin), pdisplay (Puro), pIRES (neomycin), pIRES Puro2, pQCXIP (puro), pQCXIN (neomycin), and pQCXIH (hygro).

**[0058]** Because the expression vectors can encode the fusion proteins and the FcR $\gamma$  chain together in one continuous sequence, the coding sequence can be under the control of a single promoter. Alternatively, the expression vectors can encode the fusion proteins and the FcR $\gamma$  chain under the control of separate promoters.

#### 40 Cells

**[0059]** Cell lines that may be used to express the fusion proteins of the present invention, and thus serve as the biosensor cells of the invention, are limited only in that they can stably express the fusion proteins on the surface of the cell as an integral membrane protein and that activation of the signaling domain can be detected. Suitable cell lines include, but are not limited to, lymphocytes and non-lymphoid cells.

**[0060]** The disclosure thus includes cells that stably express one or more of the fusion proteins defined herein on their surface. In some instances these cells are termed "biosensor cells" herein. In particular embodiments, the invention includes biosensor cells stably expressing on their surface more or more of the Fc $\gamma$ RI/Ig $\alpha$  fusion protein set forth in SEQ ID NO:8, the Fc $\gamma$ RIII/Ig $\alpha$  fusion protein set forth in SEQ ID NO:10, the Fc $\gamma$ RI/membrane Ig fusion protein set forth in SEQ ID NO:22, the Fc $\gamma$ RIII/membrane Ig fusion protein as set forth in SEQ ID NO:23, and a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:8, 10, 22, or 23. The cells used to prepare the biosensor cells may be any of the cells defined herein.

#### 55 Lymphocytes

**[0061]** Lymphocytes expressing the CD8/Ig $\alpha$  fusion protein have been used to demonstrate that cross-linking with an anti-CD8 antibody stimulates the release of intracellular Ca<sup>2+</sup> and phosphorylation of Ig $\alpha$  in both B and T cells [5,6,10]. Mouse and human B cell lines, which normally signal using the endogenous Ig $\alpha$ /Ig $\beta$  pathway, are particularly useful in

expression of the fusion proteins described herein. Suitable B cell lines that may be used in the production of the biosensor cells include, but are not limited to, Ramos, Raji, IIA1.6 and C604 cells lines. Other suitable B cell lines include A20 and LK 35.2.

**[0062]** Proper expression of constructs encoding any of the fusion proteins of the disclosure can be confirmed using fluorescently-labeled antibodies and flow cytometry. Cells may be cloned using limiting dilution, and selected based on their flow cytometry expression profiles for subsequent study.

**[0063]** Some B cell lines express the Fc $\gamma$ IIb inhibitory receptor, though others, such as the Ramos and IIA1.6 B cells, do not express the protein on their cell surface [11,12]. If the inhibitory activity of the Fc $\gamma$ IIb receptor is problematic in a particular cell line, siRNA constructs can be used to stably inhibit expression of Fc $\gamma$ RIIb in the cells [13] or CRISPR/Cas9 technology can be used to knockout the *Fc $\gamma$ RIIb* gene in these cell lines [14].

**[0064]** T cells expressing CD8 fused to an Ig $\alpha$  signaling domain release Ca<sup>2+</sup> after cross-linking with anti CD8 antibodies [5], which indicates that the signaling machinery in T cells can also operate through the Ig $\alpha$ . Therefore, the fusion proteins of the disclosure can also be expressed in mouse or human T cells. Suitable T cell lines that may be used in the production of the biosensor cells include, but are not limited to, Jurkat, DO-11.10 and BW5147 cell lines. Monocytes (e.g., the U937 cell line), macrophages, myoblasts (e.g., the KG1 cell line), and erythroblasts (e.g., the K562 cell line) expressing the fusion proteins may also be used as biosensor cells. Since these cells do not naturally express Fc $\gamma$ Rs, there will not be any inhibition caused by the inhibitory Fc $\gamma$ RIIb. Proper expression can also be determined using fluorescently-labeled mAbs for the Fc $\gamma$ R using flow cytometry.

#### 20 *Non-Lymphoid Cells*

**[0065]** There are a large number of established and well-characterized non-lymphoid cell lines commonly used in assays involving cell surface expression of selected proteins, such as HEK293, CHO, P815, K562, and Cos-1 cells. These cell lines are routinely used to express foreign proteins because it is easy to establish stable expression in these cells, and they have well defined growth characteristics. However, non-lymphoid cells fail to express the FcR gamma chain (FcR $\gamma$ -chain) which is a secondary protein expressed in Fc $\gamma$  receptor expressing cells. The FcR $\gamma$ -chain is required for Fc $\gamma$  receptor signaling [7]. Although non-lymphoid cells do not express the FcR- $\gamma$  chain, such cells can still serve as excellent candidates for fusion protein expression and be used as biosensor cells of the invention if they are engineered to co-express the FcR- $\gamma$  chain.

**[0066]** Non-lymphoid cells can be engineered to express the FcR- $\gamma$  chain through techniques well known to the skilled artisan. One convenient technique is to include the gene encoding the FcR- $\gamma$  chain on the constructs encoding the fusion proteins of the disclosure, where the two coding sequences are under the control of the same or separate promoters. Another convenient technique is to place expression of the fusion protein and the FcR- $\gamma$  chain under the control of the same promoter. In particular, two additional elements can be added to the constructs encoding the fusion proteins. The first element is the FcR- $\gamma$  chain itself (SEQ ID NO:25). As the FcR- $\gamma$  chain needs to be able to adopt the correct conformation in the cell membrane, it cannot be a part of the fusion protein. The second element addresses this problem as it is an engineered 2A peptide, a readily cleavable peptide first described in foot-and-mouth disease virus [8]. A variant of the original 2A peptide found in the porcine Teschovirus that cleaves more efficiently in a wide variety of cells tested [9] is used herein (SEQ ID NO:24). The FcR- $\gamma$  chain can thus be provided to non-lymphoid cells by engineering constructs encoding the fusion proteins of the disclosure to include the 2A peptide sequence C-terminal of the signaling domain, following by the FcR- $\gamma$  chain (see constructs E and F in Fig. 1).

**[0067]** Non-lymphoid cell lines that may be used in the production of the biosensor cells of the disclosure include, but are not limited to, HEK293, CHO, P815, K562, and Cos-1 cell lines.

#### 45 Antibodies

**[0068]** As will be apparent from the discussion herein, the identity of an antibody that can be used with the biosensor cells of the disclosure in the detection of target agents is only limited in that (i) the antibody can be bound by the fusion proteins of the disclosure and (ii) the antibody can bind to a target agent. Once a particular target agent is selected for detection, one can readily determine whether an antibody with binding specificity for the agent is commercially available. If it is not, an antibody with the needed binding specificity can be generated using routine methods.

**[0069]** As will be apparent, the antibodies can be monoclonal or polyclonal. The antibodies can be recombinant. Suitable antibodies also include fragments that retain the binding specificity of the antibody from which they are derived, such as, but are not limited to, Fab fragments, F(ab')<sub>2</sub> fragments, and single chain Fv (scFv) antibodies.

**[0070]** The antibodies can be conjugated to detectable labels including, but not limited to, an enzyme (e.g., peroxidase, alkaline phosphatase, glucose oxidase), a metal (e.g., gold for electron microscopy applications), a fluorescent marker (e.g., for immunofluorescence and flow cytometry applications, including CYE dyes, fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine), a fluorescence-emitting met-

als (e.g.,  $^{152}\text{Eu}$ ), a radioactive marker (e.g., radioisotopes for diagnostic purposes, including  $^3\text{H}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , and  $^{125}\text{I}$ ), a chemiluminescent marker (e.g., luminol, luciferin, isoluminol, therromatic acridinium ester, imidazole, acridinium salt and oxalate ester), and a protein tag (e.g., biotin, phycobiliprotein, c-Myc, HA, VSV-G, HSV, FLAG, V5, or HIS).

5 **[0071]** The antibodies can also be conjugated to or coated on moieties that can be used for the isolation/separation of the antibodies from a sample after they are exposed to a target agent. Such moieties include, but are not limited to, magnetic beads, agarose beads, and polystyrene beads of various diameters.

#### Samples

10 **[0072]** The samples that may be screened for the presence of a target agent are similarly limited only in that they permit binding of a target agent present in the sample by an antibody. Suitable samples include, but are not limited to, air samples, liquid samples, dry samples, vegetable samples, and biological samples. Suitable air samples include, but are not limited to, an aerosol, an atmospheric sample, a ventilator discharge, and an engine exhaust. Suitable liquid samples include, but are not limited to, a food, a drink, a water sample, a pharmaceutical formulation, and a personal care product. Suitable dry samples include, but are not limited to, a food, soil, a pharmaceutical formulation, solubilized swab samples, and a personal care product. Suitable vegetable samples include, but are not limited to, leaves, fruit, nuts, seeds, flowers, and plant tissue. Suitable biological samples include, but are not limited to, blood, serum, sweat, urine, cerebrospinal fluid, mucus, semen, stool, bronchoalveolar lavage fluid, and tissue.

#### Agents

20 **[0073]** The biosensors of the present disclosure can be used to detect a wide variety of different target agents. As will be apparent to the skilled artisan, the only limitation on the target agent is that binding of the agent by an antibody must be possible. Target agents include those of biologic origin, such as, but not limited to, bio-warfare agents, allergens, parasitic antigens, fungal antigens, viral antigens, bacterial antigens, cellular antigens, and antibodies. Exemplary bio-warfare agents include, but are not limited to, ricin, anthrax spores, botulinum toxin, *Clostridium perfringens* toxin, saxitoxin, and trichothecene mycotoxins. Exemplary allergens include, but are not limited to, tree nuts, peanuts, and animal dander. Exemplary cellular antigens include, but are not limited to, antigens associated with a disease or condition in a subject, such as a human, primate or other mammal, such as, but not limited to, livestock or a companion animal, such a dog or cat. Target agents also include plant and crop agents, aquatic pathogens or disease causing agents, drugs and other chemical compounds, and molecules found in the environment such as, but not limited to, toxins and pollutants.

#### Detecting Cellular Activation

35 **[0074]** The biosensor cells of the disclosure can be used in assays to detect, and in some cases quantify, a target agent in a sample. As described above, upon binding of the agent by antibodies, and antibody binding by the fusion proteins expressed by the biosensor cells, cross-linking occurs on the surface of the cell and the signaling domain of the fusion protein transmits the binding as activation signal within the cell. As an example, when an antigen-containing sample interacts with the antibody on the extracellular surface of the biosensor, an intracellular signaling cascade is activated.

40 **[0075]** *In vivo*, antigen receptors (membrane-bound Ig) of B cell are non-covalently associated with a disulfide-linked transmembrane heterodimer of  $\text{Ig}\alpha$  and  $\text{Ig}\beta$  proteins [16]. After cross-linking of the B cell receptor upon antigen binding, several proteins are phosphorylated on tyrosine residues by protein kinases, including  $\text{Ig}\alpha$  and  $\text{Ig}\beta$  [17,18]. One of the first downstream events after phosphorylation is  $\text{Ca}^{2+}$  release from intracellular stores followed by an influx of exogenous  $\text{Ca}^{2+}$  through  $\text{Ca}^{2+}$  channels in the cell membrane [19]. Such a change in intracellular calcium levels is one type of cellular activation contemplated herein that can be assayed. Changes in intracellular  $\text{Ca}^{2+}$  levels can be readily detected in cells by various chemical fluorescent compounds that can be efficiently loaded into cells.

45 **[0076]** Owing to the importance of  $\text{Ca}^{2+}$  in biology, numerous techniques for analyzing cellular  $\text{Ca}^{2+}$  activity have been established, which may be used in assaying cellular activation in the biosensor cells of the disclosure. A popular method is the use of fluorescent chemical  $\text{Ca}^{2+}$  indicator probes because their signal is quite large for a given change in intracellular  $\text{Ca}^{2+}$  concentration compared with other indicator types [20]. For example, cellular activation may be monitored and assayed in the biosensor cells of the description by loading the biosensor cells with Fluo-4AM, a methyl ester of Fluo-4, which is a sensitive non-ratiometric compound used to measure  $\text{Ca}^{2+}$  concentrations inside living cells [21]. Most chemical fluorescent indicators are not membrane permeant. However, the methyl ester form of Fluo-4 can passively diffuse across the plasma membrane, and once inside the cell, intracellular esterases cleave the methyl ester group off of the probe leading to a membrane-impermeant probe. Another probe alternative for use with the cells of the disclosure is Fura 2, which is a UV-excited  $\text{Ca}^{2+}$  indicator that allows ratiometric  $\text{Ca}^{2+}$  measurement. Upon binding of the target

agent by antibodies, a signal is transduced to the signaling domain of the biosensor cells which triggers the noted changes in  $\text{Ca}^{2+}$  levels which can, in turn, be assayed and/or quantified using a spectrometer to measure changes in cellular fluorescence.

[0077] Also,  $\text{Ca}^{2+}$  binding photoproteins can generate bioluminescence, which is the production of light from biological processes. Several  $\text{Ca}^{2+}$ -binding photoproteins (e.g., aequorin, obelin, mitrocomin, and clytin) have been used to measure intracellular  $\text{Ca}^{2+}$  concentration [24], each of which may be used with the biosensor cells of the disclosure to assay changes in cellular activation. The luminescence of these photoproteins upon  $\text{Ca}^{2+}$  binding is in the visible spectrum, which offers simplicity in terms of instrumentation or detection, and they are not affected by photobleaching.

[0078] It should be noted that while target agent binding (i.e., cellular activation) is exemplified herein based on measuring changes in  $\text{Ca}^{2+}$  levels in cells, other means can be used to assay for changes in target agent binding, including luminescence using photoproteins.

### III. Examples

#### Example 1: Production and Expression of Constructs Encoding Fusion Proteins

[0079] Commercially available murine  $\text{Fc}\gamma\text{RI}$  and  $\text{Ig}\alpha$  cDNAs were obtained. PCR primers providing overlapping sequence of the two genes were used to sew the two sequences together, resulting in a  $\text{Fc}\gamma\text{RI}/\text{Ig}\alpha$  in frame fusion that was confirmed by sequence analysis. Alternatively, the antibody-binding and/or transmembrane domains of  $\text{Fc}\gamma\text{RI}$  are amplified with primers from cDNA encoding the receptor, and the intracellular signaling domain of  $\text{Ig}\alpha$  is similarly amplified.

[0080] Amplified fragments are gel-purified. Amplification products (e.g.,  $\text{Fc}\gamma\text{RI}$  and  $\text{Ig}\alpha$ ) are mixed together and denatured by boiling for 5 minutes and placed at room temperature for 30 minutes prior to amplification to create a sequence encoding the full-length fusion proteins. These sequences are gel-purified and cloned into an expression vector containing a suitable promoter (e.g., a plasmid for expressing cDNA in mammalian cells), transfected into selected cell lines using Lipofectamine LX or other suitable transfection reagent, and selected using a suitable selectable marker. Individual clones are sequenced to confirm that the proper fusion protein is being expressed. Proper surface expression of the fusion proteins is determined using labeled anti-Fc receptor antibodies (e.g., anti-CD64 antibody staining) and flow cytometry. An exemplary construct encoding the  $\text{Fc}\gamma\text{RI}-\text{Ig}\alpha$  fusion protein is one encoding the antibody-binding and transmembrane domains of  $\text{Fc}\gamma\text{RI}$  (SEQ ID NO:19) and sequence encoding the  $\text{Ig}\alpha$  signaling domain (SEQ ID NO:21) in the 5' to 3' direction.

[0081] Another exemplary construct encoding the  $\text{Fc}\gamma\text{RIII}-\text{Ig}\alpha$  fusion protein is one encoding the antibody-binding and transmembrane domains of  $\text{Fc}\gamma\text{RIII}$  (SEQ ID NO:20) and sequence encoding the  $\text{Ig}\alpha$  intracellular signaling domain (SEQ ID NO:21) in the 5' to 3' direction. A commercially obtained murine  $\text{Fc}\gamma\text{RIII}$  cDNA and  $\text{Ig}\alpha$  cDNA PCR primers providing overlaps of the two genes were used to sew the two sequences together. The product resulted in a  $\text{Fc}\gamma\text{RIII}/\text{Ig}\alpha$  in frame fusion that was confirmed by sequence analysis.

[0082] An alternative approach was used to put the  $\text{Fc}\gamma\text{R}$  receptors together with the 2A peptide and  $\text{FcR}-\gamma$  chain to produce the constructs shown as E and F in Fig 1. PCR amplification with overlap extension was used to fuse 2A sequence with the  $\text{FcR}-\gamma$  chain and restriction sites were placed at the ends of the 2A and  $\text{FcR}-\gamma$ -chain cDNAs. Using PCR both the  $\text{Fc}\gamma\text{RI}$  and  $\text{Fc}\gamma\text{RIII}$  cDNAs were amplified with primers containing restriction sites on their ends that could be used to link the  $\text{Fc}\gamma\text{Rs}$  to the 2A site and for subsequent cloning into an expression vector. DNA was digested with restriction endonucleases and the products eluted from a gel. The fragments were ligated and cloned into an expression vector and they were sequenced.

[0083] The following examples provide some of the instances in which the universal biosensor cells of the disclosure can be used in practice. These examples are only a small subset of possible ways in which the biosensor can be utilized. The biosensor can be easily adapted for single or multi-well assay formats. It should be noted that the combination of cell line, construct, and  $\text{Ca}^{2+}$  indicator can vary depending on the agent, antigen or pathogen being studied and availability of antibody isotypes, and may need to be empirically determined.

#### Example 2: Detection of a Plant Virus From Leaf or Root Samples

[0084] Plant pathogens, whether viral or bacterial, are of great concern as infection and resulting loss of food and fodder crops impact the economy and food security. Therefore it is important to have assays in place that can detect routine as well as emerging plant pathogens to aid in crop management and monitoring of imported crops. The testing of domestic crops at an agricultural farm is described.

[0085] Leaf or root samples are collected from a suspected plant. The samples are thoroughly ground up to release any virus particles contained within the sample. Then magnetic beads coated with a commercially available virus-specific antibody are mixed with the sample matrix to capture the virus particles (i.e., target agents). The beads can be magnetically separated from the plant sample, thoroughly washed, and incubated with universal biosensor cells of the disclosure.

**[0086]** For example, Ramos B cells expressing either the Fc $\gamma$ RI/Ig $\alpha$  or Fc $\gamma$ RIII/Ig $\alpha$  fusion protein from a construct also encoding the FcR- $\gamma$  chain (i.e., constructs E and F of Fig. 1) may be used. Selected biosensor cells are grown to a high density (approximately 10<sup>6</sup> cells/mL) and the growth media is replaced with phenol red-free osmotically-balanced salt solution (i.e., HBSS, PBS). The cells are loaded for approximately 30-60 minutes in a Fluo-4 AM solution (approximately 2-9  $\mu$ M) in the presence of probenecid (approximately 1-2.5 mM). Probenecid is used to minimize indicator leaking from cells. Cells are thoroughly washed to remove residual Ca<sup>2+</sup> indicator. About 1-5x10<sup>6</sup> Fluo-4 AM-loaded cells in a small volume of HBSS with probenecid are transferred to multiple wells of a 96-well plate with dark sides. The plate containing the cells is then inserted into a fluorescence plate reader.

**[0087]** Several wells containing loaded cells are optically measured at 535 nm for a short period of time to establish baseline background fluorescence levels. To ensure that the cells are loaded with Fluo-4 AM, into those wells, pharmacological compounds (i.e., ATP at approximately 100-200  $\mu$ M, carbachol at approximately 30-60  $\mu$ M, or ionomycin at approximately 0.1-2  $\mu$ M) are added to stimulate an increase in intracellular Ca<sup>2+</sup> levels. Other controls, such as the use of Fc $\gamma$ R antibodies with a cross-linking secondary antibody, are used to confirm indicator loading as well.

**[0088]** After confirming Fluo-4 loading, wells containing loaded cells are incubated with a commercially available virus-specific antibody (of an isotype compatible with the construct used and ideally different from the one used for the capture beads) for approximately 30-60 minutes. Then a dilution series of the virus-coated capture beads is added to the cells and changes in fluorescence is measured over a period of several minutes. Cells are also tested with both positive controls (addition of a defined virus-containing solution) and negative controls (addition of a similar solution without virus, or addition of a solution of an irrelevant antigen that does not cross-react) to ensure specificity of the signal. Increases in cellular fluorescence indicate that the selected virus is present in the sample. In some instances, the amount of change in cellular fluorescence is correlated with the amount of selected virus present in the sample, thereby permitting quantification of the amount of the virus in the sample.

### **Example 3: Detection of Salmonella from Swab Samples**

**[0089]** *Salmonella spp.* is one of the most common food-borne pathogens and can cause serious, sometimes fatal, salmonellosis disease in young children, the elderly, and others with weakened immune systems. As *Salmonella* contamination arises from contact with tainted animal or human feces, a wide-range of foods can become contaminated from eggs and meats to produce and even water. Current *Salmonella* detection methods involve PCR or bacterial culture, which is time consuming and requires specialized knowledge. A simple, rapid detection assay is hence desirable for food quality monitoring to prevent outbreaks and product recalls. Testing for *Salmonella* in a chicken egg processing facility is described.

**[0090]** Swab samples are taken from work surfaces within the facility and exterior eggshell surfaces. The swabs are then soaked in a biocompatible solution to extract the *Salmonella* into a sample matrix that can be directly tested with the universal biosensors. In this example, C604 B cells expressing the Fc $\gamma$ RI/membrane Ig or Fc $\gamma$ RIII/membrane Ig fusion proteins (see constructs C and D of Fig. 1) are used as the biosensor cells of the disclosure. C604 cells, being B cells, will have the endogenous Ig $\alpha$  and Ig $\beta$  to provide signaling capabilities.

**[0091]** The C604 cells are grown to a high density (approximately 10<sup>6</sup> cells/mL) and media is replaced with a phenol red-free HBSS. The cells are loaded for approximately 30-60 minutes in a Fluo-4 AM solution (approximately 1-5  $\mu$ M) in the presence of probenecid (approximately 1-2.5 mM). Cells are thoroughly washed to remove residual Ca<sup>2+</sup> indicator. Between 1-5x10<sup>6</sup> Fluo-4 loaded cells in a small volume of HBSS with probenecid are transferred to multiple wells of a 96-well plate. The plate is inserted into a fluorescence plate reader and baseline background fluorescence is established. Into a subset of cell-containing wells, anti-mouse IgM (at approximately 5-7 ng/ $\mu$ L) is added to stimulate a Ca<sup>2+</sup> response as a positive control. Other controls are used to confirm loading such as the use of anti-Fc $\gamma$ RI antibodies with a secondary cross-linker antibody.

**[0092]** Commercially available anti-*Salmonella* antibody (of an isotype compatible with Fc $\gamma$ RI or Fc $\gamma$ RIII) is incubated with the cells for a period of 30-60 minutes. Then a dilution series of the *Salmonella*-containing sample is added to the cells and changes in fluorescence is measured over a period of 1-2 minutes. Cells are also tested with both positive controls and negative controls to ensure specificity of the signal. Increases in cellular fluorescence indicate the presence of *Salmonella* in the sample. In some instances, the amount of change in cellular fluorescence is correlated with the amount of *Salmonella* present in the sample, thereby permitting quantification of the amount of the *Salmonella* in the sample.

### **Example 4: Detection of Listeria From Food Samples**

**[0093]** *Listeria* (i.e., *L. monocytogenes*) is a food-borne pathogen that is the causative agent of listeriosis, a serious bacterial disease with an approximate 20% fatality rate and is most dangerous to pregnant women, infants, and those with weakened immune systems. *Listeria* can contaminate raw meats, produce, and dairy products, and prepared foods.

Hence the ability to detect the bacteria and monitor for its presence is desirable in order to prevent pathogen outbreaks and product recalls. The use of the universal biosensor cells for the detection of *Listeria* in a meat processing plant that produces ready-to-eat foods (i.e., deli meats and hot dogs) is described.

[0094] As similarly described in Example 3, the work surfaces and equipment of the plant is swabbed before, during, and after meat processing to monitor for potential contamination of the products and to assess the effectiveness of decontamination procedures. Additionally, samples of processed meats may be tested. The samples are homogenized in PBS and mixed with microscopic magnetic beads that are coated with a commercially available *Listeria*-specific antibody. The beads bind any *Listeria* present in the sample and are magnetically separated from the sample, thoroughly washed, and added to prepared universal biosensors.

[0095] COS-1 cells stably expressing either Fc $\gamma$ RI/Ig $\alpha$  or Fc $\gamma$ RIII/Ig $\alpha$  fusion proteins along with the FcR- $\gamma$  chain and the bioluminescent photoprotein aequorin are used as the biosensor cells and are grown to a high density (approximately 10<sup>6</sup> cells/mL). The cells are incubated with approximately 2-8  $\mu$ M coelenterazine (a necessary substrate of aequorin) over a period of 5-16 hours. After thorough washing to remove excess coelenterazine, cells are plated into multiple wells of a 96-well plate. Cells are then incubated with a commercially available *Listeria*-specific antibody (of an isotype compatible with the construct used and preferably a different antibody than the one used for the capture beads) for 30-60 minutes. The plate is inserted into a luminescence plate reader and a baseline background luminescence level is measured. Confirmation of successful coelenterazine loading and Ca<sup>2+</sup> responsiveness is obtained by the addition of 0.15-100  $\mu$ M ATP. After which, the *Listeria*-coated capture beads are added to the cells at differing dilutions and changes in luminescence signal are recorded over a period of 1-2 minutes. Increases in luminescence indicate the presence of *Listeria* in the sample. In some instances, the amount of change in luminescence is correlated with the amount of *Listeria* present in the sample, thereby permitting quantification of the amount of the *Listeria* in the sample.

#### **Example 5: Detection of *B. anthracis* Spores from Air Samples**

[0096] Anthrax is a rapid-onset and lethal disease caused by the spores of the bacterium *Bacillus anthracis*. A native soil bacterium, it can be transmitted through contact with infected meat from pasture-raised animals as well as unprocessed animal hides and wool. More recently, *B. anthracis* has been weaponized for use in biological warfare and in terrorist attacks. In this regard, reliable and rapid detection of *B. anthracis* spores is crucial. Test samples may be obtained by swabbing suspected areas or suspending suspected powders directly into PBS for analysis in solution. Alternatively, aerosol samples may be collected in a suspected area and particulates can be concentrated onto surfaces and exposed to universal biosensor cells. A suitable aerosol-sampling device (BioFlash E) is produced by PathSensors, Inc.

[0097] In this example, Jurkat cells, a human T cell line, expressing the Fc $\gamma$ RI/Ig $\alpha$  or Fc $\gamma$ RIII/Ig $\alpha$  fusion proteins and the FcR $\gamma$ -chain are used as the biosensor cells. The cells are loaded with Indo-1 Ca<sup>2+</sup> indicator (approximately 1-5  $\mu$ M) for a period of 30-60 minutes. After thorough washing, the cells are incubated with commercially available *B. anthracis*-specific antibodies (of an isotype compatible with the construct used) and loaded into a chamber inside of the aerosol-sampling machine. Baseline background fluorescence at 405 nm is established. Confirmation of successful Ca<sup>2+</sup> indicator loading is obtained by the addition of approximately 1-5  $\mu$ g/mL ionomycin. Then air from the monitored area is passed through the machine and particulate matter is concentrated on an interior surface. The biosensors are then released onto the test surface to bind any *B. anthracis* spores that may be present. Changes in fluorescence signal at 405 nm are recorded over a period of 1-2 minutes. Increases in cellular fluorescence indicate the presence of anthrax in the sample. In some instances, the amount of change in cellular fluorescence is correlated with the amount of anthrax present in the sample, thereby permitting quantification of the amount of the anthrax in the sample. The scope of the appended claims is not to be limited to the specific embodiments described.

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[0098] All of the following references have been cited in this application:

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5 SEQUENCE LISTING

**[0099]**

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 <223> Chimeric fusion protein construct comprising Fc-gammaRIII and Ig alpha

<400> 9

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 20 atgtgcgaag ggaccacaaa ccctgggaac tcttctactc agtggttcca caactggagt 240  
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 gaatatcggg gtcaaatgga gcagaccgc ctgagcgacc ctgtagatct gggagtgatt 360  
 25 tctgactggc tgctgctcca gaccctcag cgggtgtttc tggaagggga aaccatcacg 420  
 ctaagggtgcc ctagctggag gaacaaacta ctgaacagga tctcgttctt ccataatgaa 480  
 30 aaatccgtga ggtatcatca ctacaaaagt aatttctcta tcccaaaagc caaccacagt 540  
 cacagtgggg actactactg caaaggaagt ctaggaagta cacagcacca gtccaagcct 600  
 gtcaccatca ctgtccaaga cccagcaact acatcctcca tctctctagt ctggcaccac 660  
 35 actgctttct ccctagtgat gtgcctcctg ttgacagtgt tcaggaaacg gtggcaaaat 720  
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 aaccttgatg actgttctat gtatgaggac atctccaggg gactccaggg cacctaccag 840  
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45 <210> 10  
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 <212> PRT  
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<220>  
 <223> Chimeric fusion protein construct comprising Fc-gammaRIII and Ig alpha

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Trp Leu Leu Pro Pro Leu Thr Ile Leu Leu Leu Phe Ala Phe Ala Asp  
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Arg Gln Ser Ala Ala Leu Pro Lys Ala Val Val Lys Leu Asp Pro Pro  
 35 40 45  
 5 Trp Ile Gln Val Leu Lys Glu Asp Met Val Thr Leu Met Cys Glu Gly  
 50 55 60  
 10 Thr His Asn Pro Gly Asn Ser Ser Thr Gln Trp Phe His Asn Trp Ser  
 65 70 75 80  
 15 Ser Ile Arg Ser Gln Val Gln Ser Ser Tyr Thr Phe Lys Ala Thr Val  
 85 90 95  
 20 Asn Asp Ser Gly Glu Tyr Arg Cys Gln Met Glu Gln Thr Arg Leu Ser  
 100 105 110  
 25 Asp Pro Val Asp Leu Gly Val Ile Ser Asp Trp Leu Leu Leu Gln Thr  
 115 120 125  
 30 Pro Gln Arg Val Phe Leu Glu Gly Glu Thr Ile Thr Leu Arg Cys Pro  
 130 135 140  
 35 Ser Trp Arg Asn Lys Leu Leu Asn Arg Ile Ser Phe Phe His Asn Glu  
 145 150 155 160  
 40 Lys Ser Val Arg Tyr His His Tyr Lys Ser Asn Phe Ser Ile Pro Lys  
 165 170 175  
 45 Ala Asn His Ser His Ser Gly Asp Tyr Tyr Cys Lys Gly Ser Leu Gly  
 180 185 190  
 50 Ser Thr Gln His Gln Ser Lys Pro Val Thr Ile Thr Val Gln Asp Pro  
 195 200 205  
 55 Ala Thr Thr Ser Ser Ile Ser Leu Val Trp His His Thr Ala Phe Ser  
 210 215 220  
 60 Leu Val Met Cys Leu Leu Phe Ala Val Phe Arg Lys Arg Trp Gln Asn  
 225 230 235 240  
 65 Glu Lys Phe Gly Val Asp Met Pro Asp Asp Tyr Glu Asp Glu Asn Leu  
 245 250 255  
 70 Tyr Glu Gly Leu Asn Leu Asp Asp Cys Ser Met Tyr Glu Asp Ile Ser  
 260 265 270  
 75 Arg Gly Leu Gln Gly Thr Tyr Gln Asp Val Gly Asn Leu His Ile Gly  
 275 280 285

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Asp Ala Gln Leu Glu Lys Pro  
290 295

5 <210> 11  
 <211> 1752  
 <212> DNA  
 <213> Artificial Sequence

10 <220>  
 <223> Chimeric fusion protein construct comprising Fc-gammaRI and membrane Ig

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 gtcagtattt tccagaagga aatgtcact ttatggtgtg aggggcctca cctgcctgga 180  
 20 gacagttcca cacaatggtt tatcaacgga acagccgttc agatctccac gcctagttat 240  
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 atgccaagtg accctgtgca gttgcaaatc cacaatgatt ggctgctact ccaggcctcc 360  
 25 cgcagagtcc tcacagaag agaacccctg gccttgaggt gtcacggatg gaagaataaa 420  
 ctggtgtaca atgtggtttt ctatagaaat ggaaaatcct ttcagttttc ttcagattcg 480  
 30 gaggtcgcca ttctgaaaac caacctgagt cacagcggca tctaccactg ctcaggcacg 540  
 ggaagacacc gctacacatc tgcaggagtg tccatcacgg tgaaagagct gtttaccacg 600  
 ccagtgtga gagcatccgt gtcactctcc tcccggagg ggagtctggt caccctgaac 660  
 35 tgtgagacga atttgtcctt gcagagacc ggcttacagc ttcacttctc cttctacgtg 720  
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 40 cgcagccctg agttggagga gcgcaaatgt tgtgtcgagt gccaccctg cccagcacca 900  
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 cagttcaact ggtacgtgga cggcatggag gtgcataatg ccaagacaaa gccacgggag 1080  
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 50 ctgaacggca aggagtacaa gtgcaaggtc tccaacaaag gcctcccagc ccccatcgag 1200  
 aaaaccatct ccaaaaccaa agggcagccc cgagaaccac aggtgtacac cctgccccca 1260  
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 55 cccagcgaca tcgccgtgga gtgggagagc aatgggcagc cggagaacaa ctacaagacc 1380  
 acacctccca tgctggactc cgacggctcc ttcttctct acagcaagct caccgtggac 1440

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aaccactaca cacagaagag cctctccctg tctccggagc tgcaactgga ggagagctgt 1560  
5 gcggagggcg aggacgggga gctggacggg ctgtggacga ccatcaccat cttcatcaca 1620  
ctcttcctgc taagcgtgtg ctacagtgcc accatcacct tcttcaaggt gaagtggatc 1680  
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<211> 583

<212> PRT

<213> Artificial Sequence

<220>

<223> Chimeric fusion protein construct comprising Fc-gammaRI and membrane Ig

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Val Ile Thr Leu Gln Pro Pro Trp Val Ser Ile Phe Gln Lys Glu Asn  
35 40  
Val Thr Leu Trp Cys Glu Gly Pro His Leu Pro Gly Asp Ser Ser Thr  
50 55 60  
Gln Trp Phe Ile Asn Gly Thr Ala Val Gln Ile Ser Thr Pro Ser Tyr  
65 70 75 80  
Ser Ile Pro Glu Ala Ser Phe Gln Asp Ser Gly Glu Tyr Arg Cys Gln  
85 90 95  
45 Ile Gly Ser Ser Met Pro Ser Asp Pro Val Gln Leu Gln Ile His Asn  
100 105 110  
50 Asp Trp Leu Leu Leu Gln Ala Ser Arg Arg Val Leu Thr Glu Gly Glu  
115 120 125  
Pro Leu Ala Leu Arg Cys His Gly Trp Lys Asn Lys Leu Val Tyr Asn  
130 135 140  
55 Val Val Phe Tyr Arg Asn Gly Lys Ser Phe Gln Phe Ser Ser Asp Ser  
145 150 155 160

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Glu Val Ala Ile Leu Lys Thr Asn Leu Ser His Ser Gly Ile Tyr His  
 165 170 175  
 5 Cys Ser Gly Thr Gly Arg His Arg Tyr Thr Ser Ala Gly Val Ser Ile  
 180 185 190  
 Thr Val Lys Glu Leu Phe Thr Thr Pro Val Leu Arg Ala Ser Val Ser  
 10 195 200 205  
 Ser Pro Phe Pro Glu Gly Ser Leu Val Thr Leu Asn Cys Glu Thr Asn  
 15 210 215 220  
 Leu Leu Leu Gln Arg Pro Gly Leu Gln Leu His Phe Ser Phe Tyr Val  
 20 225 230 235 240  
 Gly Ser Lys Ile Leu Glu Tyr Arg Asn Thr Ser Ser Glu Tyr His Ile  
 25 245 250 255  
 Ala Arg Ala Glu Arg Glu Asp Ala Gly Phe Tyr Trp Cys Glu Val Ala  
 30 260 265 270  
 Thr Glu Asp Ser Ser Val Leu Lys Arg Ser Pro Glu Leu Glu Glu Arg  
 35 275 280 285  
 Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly  
 40 290 295 300  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 45 305 310 315 320  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 50 325 330 335  
 Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu Val His  
 55 340 345 350  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg  
 355 360 365  
 Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys  
 370 375 380  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu  
 385 390 395 400  
 Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr

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5	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu
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10	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp
			435					440					445			
15	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Met
		450					455					460				
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	465					470					475					480
25	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His
					485					490					495	
30	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro
				500					505					510		
35	Glu	Leu	Gln	Leu	Glu	Glu	Ser	Cys	Ala	Glu	Ala	Gln	Asp	Gly	Glu	Leu
			515					520					525			
40	Asp	Gly	Leu	Trp	Thr	Thr	Ile	Thr	Ile	Phe	Ile	Thr	Leu	Phe	Leu	Leu
		530					535					540				
45	Ser	Val	Cys	Tyr	Ser	Ala	Thr	Ile	Thr	Phe	Phe	Lys	Val	Lys	Trp	Ile
	545					550					555					560
50	Phe	Ser	Ser	Val	Val	Asp	Leu	Lys	Gln	Thr	Ile	Val	Pro	Asp	Tyr	Arg
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45 <210> 13  
 <211> 1515  
 <212> DNA  
 <213> Artificial Sequence

50 <220>  
 <223> Chimeric fusion protein construct comprising Fc-gammaRIII and membrane Ig

<400> 13

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 5 gctgtgggga aactggaccc cccatggatc caggtgctca aggaagacat ggtgacactg 180  
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 gaatatcggg gtcaaatgga gcagaccgac ctgtagatct gggagtgatt 360  
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 ccacctgtgg caggaccgtc agtcttctc tccccccaa aaccaagga caccctcatg 720  
 atctcccgga cccctgaggt cacgtgcgtg gtgggtggacg tgagccacga agaccccag 780  
 25 gtccagttca actggtacgt ggacggcatg gaggtgcata atgccaagac aaagccacgg 840  
 gaggagcagt tcaacagcac gttccgtgtg gtcagcgtcc tcaccgtcgt gcaccaggac 900  
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 40 gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg 1260  
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10

15

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45

50

55

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	Trp	Ile	Gln	Val	Leu	Lys	Glu	Asp	Met	Val	Thr	Leu	Met	Cys	Glu	Gly
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	Thr	His	Asn	Pro	Gly	Asn	Ser	Ser	Thr	Gln	Trp	Phe	His	Asn	Trp	Ser
	65					70					75					80
15	Ser	Ile	Arg	Ser	Gln	Val	Gln	Ser	Ser	Tyr	Thr	Phe	Lys	Ala	Thr	Val
					85					90					95	
	Asn	Asp	Ser	Gly	Glu	Tyr	Arg	Cys	Gln	Met	Glu	Gln	Thr	Arg	Leu	Ser
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	Asp	Pro	Val	Asp	Leu	Gly	Val	Ile	Ser	Asp	Trp	Leu	Leu	Leu	Gln	Thr
			115					120					125			
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	Pro	Gln	Arg	Val	Phe	Leu	Glu	Gly	Glu	Thr	Ile	Thr	Leu	Arg	Cys	Pro
		130					135					140				
30	Ser	Trp	Arg	Asn	Lys	Leu	Leu	Asn	Arg	Ile	Ser	Phe	Phe	His	Asn	Glu
	145					150					155					160
	Lys	Ser	Val	Arg	Tyr	His	His	Tyr	Lys	Ser	Asn	Phe	Ser	Ile	Pro	Lys
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	Ala	Asn	His	Ser	His	Ser	Gly	Asp	Tyr	Tyr	Cys	Lys	Gly	Ser	Leu	Gly
				180					185					190		
40																
	Ser	Thr	Gln	His	Gln	Ser	Lys	Pro	Val	Thr	Ile	Thr	Val	Gln	Asp	Glu
			195					200					205			
45	Arg	Lys	Cys	Cys	Val	Glu	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Pro	Val	Ala
		210					215					220				
	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met
50						230					235					240
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					245					250					255	
55	Glu	Asp	Pro	Glu	Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Met	Glu	Val
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 290 295 300  
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 Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val  
 325 330 335  
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 Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser  
 340 345 350  
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 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
 355 360 365  
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 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 370 375 380  
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 Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
 385 390 395 400  
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 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
 405 410 415  
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 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
 420 425 430  
 50  
 Pro Glu Leu Gln Leu Glu Glu Ser Cys Ala Glu Ala Gln Asp Gly Glu  
 435 440 445  
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 Leu Asp Gly Leu Trp Thr Thr Ile Thr Ile Phe Ile Thr Leu Phe Leu  
 450 455 460  
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 Leu Ser Val Cys Tyr Ser Ala Thr Ile Thr Phe Phe Lys Val Lys Trp  
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<210> 15  
<211> 1215

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<212> DNA  
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5	cta ctt tgg gtt cca gtc ggt ggg gaa gtg gtt aat gcc acc aag gct	96
	Leu Leu Trp Val Pro Val Gly Gly Glu Val Val Asn Ala Thr Lys Ala	
	20 25 30	
10	gtg atc acc ttg cag cct cca tgg gtc agt att ttc cag aag gaa aat	144
	Val Ile Thr Leu Gln Pro Pro Trp Val Ser Ile Phe Gln Lys Glu Asn	
	35 40 45	
15	gtc act tta tgg tgt gag ggg cct cac ctg cct gga gac agt tcc aca	192
	Val Thr Leu Trp Cys Glu Gly Pro His Leu Pro Gly Asp Ser Ser Thr	
	50 55 60	
20	caa tgg ttt atc aac gga aca gcc gtt cag atc tcc acg cct agt tat	240
	Gln Trp Phe Ile Asn Gly Thr Ala Val Gln Ile Ser Thr Pro Ser Tyr	
	65 70 75 80	
25	agc atc cca gag gcc agt ttt cag gac agt ggc gaa tac agg tgt cag	288
	Ser Ile Pro Glu Ala Ser Phe Gln Asp Ser Gly Glu Tyr Arg Cys Gln	
	85 90 95	
30	ata ggt tcc tca atg cca agt gac cct gtg cag ttg caa atc cac aat	336
	Ile Gly Ser Ser Met Pro Ser Asp Pro Val Gln Leu Gln Ile His Asn	
	100 105 110	
35	gat tgg ctg cta ctc cag gcc tcc cgc aga gtc ctc aca gaa gga gaa	384
	Asp Trp Leu Leu Leu Gln Ala Ser Arg Arg Val Leu Thr Glu Gly Glu	
	115 120 125	
40	ccc ctg gcc ttg agg tgt cac gga tgg aag aat aaa ctg gtg tac aat	432
	Pro Leu Ala Leu Arg Cys His Gly Trp Lys Asn Lys Leu Val Tyr Asn	
	130 135 140	
45	gtg gtt ttc tat aga aat gga aaa tcc ttt cag ttt tct tca gat tcg	480
	Val Val Phe Tyr Arg Asn Gly Lys Ser Phe Gln Phe Ser Ser Asp Ser	
	145 150 155 160	
50	gag gtc gcc att ctg aaa acc aac ctg agt cac agc ggc atc tac cac	528
	Glu Val Ala Ile Leu Lys Thr Asn Leu Ser His Ser Gly Ile Tyr His	
	165 170 175	
55	tgc tca ggc acg gga aga cac cgc tac aca tct gca gga gtg tcc atc	576
	Cys Ser Gly Thr Gly Arg His Arg Tyr Thr Ser Ala Gly Val Ser Ile	
	180 185 190	
60	acg gtg aaa gag ctg ttt acc acg cca gtg ctg aga gca tcc gtg tca	624
	Thr Val Lys Glu Leu Phe Thr Thr Pro Val Leu Arg Ala Ser Val Ser	
	195 200 205	
65	tct ccc ttc ccg gag ggg agt ctg gtc acc ctg aac tgt gag acg aat	672
	Ser Pro Phe Pro Glu Gly Ser Leu Val Thr Leu Asn Cys Glu Thr Asn	
	210 215 220	

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	Gly Ser Lys Ile Leu Glu Tyr Arg Asn Thr Ser Ser Glu Tyr His Ile	
	245 250 255	
10	gca agg gcg gaa aga gaa gat gct gga ttc tac tgg tgt gag gta gcc	816
	Ala Arg Ala Glu Arg Glu Asp Ala Gly Phe Tyr Trp Cys Glu Val Ala	
	260 265 270	
15	acg gag gac agc agt gtc ctt aag cgc agc cct gag ttg gag ctc caa	864
	Thr Glu Asp Ser Ser Val Leu Lys Arg Ser Pro Glu Leu Glu Leu Gln	
	275 280 285	
20	gtg ctt ggt ccc cag tca tca gct cct gtc tgg ttt cac atc ctg ttt	912
	Val Leu Gly Pro Gln Ser Ser Ala Pro Val Trp Phe His Ile Leu Phe	
	290 295 300	
25	tat ctg tca gtg gga ata atg ttt tcg ttg aac acg gtt ctc tat gtg	960
	Tyr Leu Ser Val Gly Ile Met Phe Ser Leu Asn Thr Val Leu Tyr Val	
	305 310 315 320	
30	aaa ata cac agg ctg cag aga gag aag aaa tac aac tta gaa gtc cct	1008
	Lys Ile His Arg Leu Gln Arg Glu Lys Lys Tyr Asn Leu Glu Val Pro	
	325 330 335	
35	ttg gtt tct gag cag gga aag aaa gca aat tcc ttt cag caa gtt aga	1056
	Leu Val Ser Glu Gln Gly Lys Lys Ala Asn Ser Phe Gln Gln Val Arg	
	340 345 350	
40	agc gat ggc gtg tat gaa gaa gta aca gcc act gcg agc cag acc aca	1104
	Ser Asp Gly Val Tyr Glu Glu Val Thr Ala Thr Ala Ser Gln Thr Thr	
	355 360 365	
45	cca aaa gaa gcg ccc gat gga cct cga agc tca gtg ggt gac tgt gga	1152
	Pro Lys Glu Ala Pro Asp Gly Pro Arg Ser Ser Val Gly Asp Cys Gly	
	370 375 380	
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	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val	
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					85					90					95		
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	Asp	Pro	Val	Asp	Leu	Gly	Val	Ile	Ser	Asp	Trp	Leu	Leu	Leu	Gln	Thr	
				115				120						125			
20	cct	cag	cgg	gtg	ttt	ctg	gaa	ggg	gaa	acc	atc	acg	cta	agg	tgc	cct	432
	Pro	Gln	Arg	Val	Phe	Leu	Glu	Gly	Glu	Thr	Ile	Thr	Leu	Arg	Cys	Pro	
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	Ala	Asn	His	Ser	His	Ser	Gly	Asp	Tyr	Tyr	Cys	Lys	Gly	Ser	Leu	Gly	
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40	agt	aca	cag	cac	cag	tcc	aag	cct	gtc	acc	atc	act	gtc	caa	gac	gag	624
	Ser	Thr	Gln	His	Gln	Ser	Lys	Pro	Val	Thr	Ile	Thr	Val	Gln	Asp	Glu	
					195			200						205			
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	Arg	Lys	Cys	Cys	Val	Glu	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Pro	Val	Ala	
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 1 5 10 15

25 gcc gcc ctg gga gag ccg cag ctc tgc tat atc ctg gat gct gtc ctg 96  
 Ala Ala Leu Gly Glu Pro Gln Leu Cys Tyr Ile Leu Asp Ala Val Leu  
 20 25 30

30 ttt ttg tat ggt att gtc ctt acc cta ctc tac tgt cga ctc aag atc 144  
 Phe Leu Tyr Gly Ile Val Leu Thr Leu Leu Tyr Cys Arg Leu Lys Ile  
 35 40 45

cag gtc cga aag gca gct ata gcc agc cgt gag aaa gca gat gct gtc 192  
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 50 55 60

35 tac acg ggc ctg aac acc cgg agc cag gag aca tat gag act ctg aag 240  
 Tyr Thr Gly Leu Asn Thr Arg Ser Gln Glu Thr Tyr Glu Thr Leu Lys  
 65 70 75 80

40 cat gag aaa cca ccc cag tag 261  
 His Glu Lys Pro Pro Gln  
 85

Claims

- 45 1. A method of detecting a target agent in a sample, comprising
- (a) contacting a sample with an antibody having binding specificity for a target agent and with a biosensor cell, and  
 (b) assaying the biosensor cell for cellular activation,
- 50 wherein the biosensor cell stably expresses a chimeric fusion protein, and wherein the chimeric fusion protein comprises an Fc $\gamma$  receptor (Fc $\gamma$ R) antibody-binding domain and a signaling domain, wherein the signaling domain is the immunoglobulin alpha (Ig $\alpha$ ) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5, or wherein the signaling domain is the membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6.
- 55 2. The method of claim 1, wherein the sample is an air sample, a liquid sample, a vegetable sample, or a dry sample,

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preferably said sample is a biological sample selected from the group consisting of blood, serum, sweat, urine, cerebrospinal fluid, mucus, semen, stool, bronchoalveolar lavage fluid, and tissue.

- 5 3. The method of claim 1, wherein the agent is an environmental toxin, pollutant, or drug, preferably said agent is a biologic agent selected from the group consisting of a bio-warfare agent, an allergen, a parasitic antigen, a fungal antigen, a viral antigen, a bacterial antigen, a cellular antigen, and an antibody.
- 10 4. The method of claim 1, wherein the biosensor cell is a B cell, a T cell, a monocyte, a macrophage, a HEK293 cell, a CHO cell, P815, K562, or a Cos-1 cell that stably expresses the chimeric fusion protein and wherein cellular activation is an increase in intracellular  $Ca^{2+}$  levels.
- 15 5. The method of any one of claims 1-4, wherein the  $Fc\gamma R$  antibody-binding domain is the  $Fc\gamma RI$  antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3, or wherein the  $Fc\gamma R$  antibody-binding domain is the  $Fc\gamma RIII$  antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4.
- 20 6. A biosensor cell stably expressing a chimeric fusion protein, wherein the chimeric fusion protein comprises an  $Fc\gamma$  receptor ( $Fc\gamma R$ ) antibody-binding domain and a signaling domain and wherein preferably the biosensor cell is a B cell, a T cell, a monocyte, a macrophage, a HEK293 cell, a CHO cell, P815, K562, or a Cos-1 cell that stably expresses the chimeric fusion protein, wherein the signaling domain is the immunoglobulin alpha ( $Ig\alpha$ ) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5 or wherein the signaling domain is the membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6.
- 25 7. The biosensor cell of claim 6, wherein the  $Fc\gamma R$  antibody-binding domain is the  $Fc\gamma RI$  antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3 or wherein the  $Fc\gamma R$  antibody-binding domain is the  $Fc\gamma RIII$  antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4.
- 30 8. A chimeric fusion protein comprising an  $Fc\gamma$  receptor ( $Fc\gamma R$ ) antibody-binding domain and a signaling domain, wherein the signaling domain is the immunoglobulin alpha ( $Ig\alpha$ ) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5, or wherein the signaling domain is the membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6.
- 35 9. The chimeric fusion protein of claim 8, wherein the  $Fc\gamma R$  antibody-binding domain is the  $Fc\gamma RI$  antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3, or wherein the  $Fc\gamma R$  antibody-binding domain is the  $Fc\gamma RIII$  antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4.
- 40 10. The chimeric fusion protein of claim 8, wherein the fusion protein is the  $Fc\gamma RI/Ig\alpha$  fusion protein set forth in SEQ ID NO:8 or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:8, or wherein the fusion protein is the  $Fc\gamma RIII/Ig\alpha$  fusion protein set forth in SEQ ID NO:10 or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:10.
- 45 11. The chimeric fusion protein of claim 8, wherein the fusion protein is the  $Fc\gamma RI$ /membrane Ig fusion protein set forth in SEQ ID NO:22 or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:22, or wherein the fusion protein is the  $Fc\gamma RIII$ /membrane Ig fusion protein set forth in SEQ ID NO:23 or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:23.
- 50 12. A polynucleotide sequence encoding a chimeric fusion protein according to any one of claims 8-11, or a complementary strand thereof.
- 55 13. A cloning vector or a cell comprising a polynucleotide sequence of claim 12.

14. A cell comprising a cloning vector of claim 13.

15. A method of producing a chimeric fusion protein comprising culturing a cell according to claim 13 or a cell according to claim 14, under conditions promoting expression of the fusion protein, and recovering the fusion protein from the cell or cell culture.

### Patentansprüche

1. Verfahren zur Erfassung eines Zielmittels in einer Probe, welches umfasst:

- (a) Inkontaktbringen einer Probe mit einem Antikörper, der eine Bindungsspezifität für ein Zielmittel aufweist, und mit einer Biosensor-Zelle, und
- (b) Überprüfen der Biosensor-Zelle auf zelluläre Aktivierung,

wobei die Biosensor-Zelle stabil ein chimäres Fusionsprotein exprimiert, und wobei das chimäre Fusionsprotein eine Fc $\gamma$ -Rezeptor (Fc $\gamma$ R) Antikörper-Bindungsdomäne und eine Signaldomäne umfasst, wobei die Signaldomäne die Immunglobulin alpha (Ig $\alpha$ ) Signaldomäne gemäß SEQ ID NR. 5 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 5, oder wobei die Signaldomäne das Membran Ig gemäß SEQ ID NR. 6 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 6.

2. Verfahren nach Anspruch 1, wobei die Probe eine Luft-Probe, eine flüssige Probe, eine pflanzliche Probe oder eine trockene Probe ist, vorzugsweise wobei die Probe eine biologische Probe ist ausgewählt aus der Gruppe bestehend aus Blut, Serum, Schweiß, Urin, zerebrospinaler Flüssigkeit, Mucus, Samen, Stuhl, bronchoalveolarer Lavageflüssigkeit, und Gewebe.

3. Verfahren nach Anspruch 1, wobei das Mittel ein Umwelttoxin ist, ein Schadstoff, oder ein Arzneimittel, vorzugsweise wobei das Mittel ein biologisches Mittel ist ausgewählt aus der Gruppe bestehend aus einem Mittel zur biologischen Kriegsführung, einem Allergen, einem Parasiten-Antigen, einem Pilz-Antigen, einem viralen Antigen, einem bakteriellen Antigen, einem zellulären Antigen und einem Antikörper.

4. Verfahren nach Anspruch 1, wobei die Biosensor-Zelle eine B-Zelle, eine T-Zelle, ein Monozyt, ein Makrophage, eine HEK293-Zelle, eine CHO-Zelle, P815, K562, oder eine Cos-1-Zelle ist, die stabil das chimäre Fusionsprotein exprimiert und wobei die zelluläre Aktivierung ein Anstieg an intrazellulärem Ca<sup>2+</sup> ist.

5. Verfahren nach einem der Ansprüche 1-4, wobei die Fc $\gamma$ R-Antikörper-Bindungsdomäne die Fc $\gamma$ RI-Antikörper-Bindungsdomäne gemäß SEQ ID NR. 1 oder 3 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 1 oder 3, oder wobei die Fc $\gamma$ R-Antikörper-Bindungsdomäne die Fc $\gamma$ RIII-Antikörper-Bindungsdomäne gemäß SEQ ID NR. 2 oder 4 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 2 oder 4.

6. Biosensor-Zelle, welche stabil ein chimäres Fusionsprotein exprimiert, worin das chimäre Fusionsprotein eine Fc $\gamma$ -Rezeptor (Fc $\gamma$ R) Antikörper-Bindungsdomäne und eine Signaldomäne umfasst und worin die Biosensor-Zelle vorzugsweise eine B-Zelle, eine T-Zelle, ein Monozyt, ein Makrophage, eine HEK293-Zelle, eine CHO-Zelle, P815, K562, oder eine Cos-1-Zelle ist, die stabil das chimäre Fusionsprotein exprimiert, worin die Signaldomäne die Immunglobulin alpha (Ig $\alpha$ ) Signaldomäne gemäß SEQ ID NR. 5 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 5 oder worin die Signaldomäne das Membran Ig gemäß SEQ ID NR. 6 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 6.

7. Biosensor-Zelle nach Anspruch 6, worin die Fc $\gamma$ R-Antikörper-Bindungsdomäne die Fc $\gamma$ RI-Antikörper-Bindungsdomäne gemäß SEQ ID NR. 1 oder 3 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 1 oder 3 oder worin die Fc $\gamma$ R-Antikörper-Bindungsdomäne die Fc $\gamma$ RIII-Antikörper-Bindungsdomäne gemäß SEQ ID NR. 2 oder 4 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 2 oder 4.

8. Chimäres Fusionsprotein, welches eine Fc $\gamma$ -Rezeptor (Fc $\gamma$ R) Antikörper-Bindungsdomäne und ein Signaldomäne

umfasst, worin die Signaldomäne die Immunglobulin alpha ( $Ig\alpha$ ) Signaldomäne gemäß SEQ ID NR. 5 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 5, oder worin die Signaldomäne das Membran Ig gemäß SEQ ID NR. 6 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 6.

5

9. Chimäres Fusionsprotein nach Anspruch 8, worin die  $Fc\gamma R$ -Antikörper-Bindungsdomäne die  $Fc\gamma RI$ -Antikörper-Bindungsdomäne gemäß SEQ ID NR. 1 oder 3 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 1 oder 3, oder worin die  $Fc\gamma R$ -Antikörper-Bindungsdomäne die  $Fc\gamma RIII$ -Antikörper-Bindungsdomäne gemäß SEQ ID NR. 2 oder 4 ist, oder eine Sequenzvariante davon mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 2 oder 4.

10

10. Chimäres Fusionsprotein nach Anspruch 8, worin das Fusionsprotein das  $Fc\gamma RVIg\alpha$ -Fusionsprotein gemäß SEQ ID NR. 8 ist oder eine Sequenzvariante mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 8, oder worin das Fusionsprotein das  $Fc\gamma RIIIVg\alpha$ -Fusionsprotein gemäß SEQ ID NR. 10 ist oder eine Sequenzvariante mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NO: 10.

15

11. Chimäres Fusionsprotein nach Anspruch 8, worin das Fusionsprotein das  $Fc\gamma RV$ -Membran Ig-Fusionsprotein gemäß SEQ ID NR. 22 ist oder eine Sequenzvariante mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 22, oder worin das Fusionsprotein das  $Fc\gamma RIIIV$ -Membran Ig-Fusionsprotein gemäß SEQ ID NR. 23 ist oder eine Sequenzvariante mit mindestens 95% Sequenzidentität über die Gesamtlänge der SEQ ID NR. 23.

20

12. Polynucleotidsequenz, welche ein chimäres Fusionsprotein nach einem der Ansprüche 8-11 codiert, oder ein komplementärer Strang davon.

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13. Klonierungsvektor oder eine Zelle, der/die eine Polynucleotidsequenz nach Anspruch 12 umfasst.

14. Zelle, welche einen Klonierungsvektor nach Anspruch 13 umfasst.

15. Verfahren zur Herstellung eines chimären Fusionsprotein, welches umfasst, Züchten einer Zelle nach Anspruch 13 oder einer Zelle nach Anspruch 14 unter Bedingungen, welche die Expression des Fusionsproteins fördern, und Gewinnen des Fusionsproteins aus der Zelle oder der Zellkultur.

30

## Revendications

35

1. Procédé de détection d'un agent cible dans un échantillon, comprenant

(a) la mise en contact d'un échantillon avec un anticorps possédant une spécificité de liaison pour un agent cible et avec une cellule de biocapteur, et

40

(b) l'analyse de la cellule de biocapteur pour une activation cellulaire,

dans lequel la cellule de biocapteur exprime stablement une protéine de fusion chimérique, et dans lequel la protéine de fusion chimérique comprend un domaine de liaison à un anticorps du récepteur  $Fc\gamma$  ( $Fc\gamma R$ ) et un domaine de signalisation, dans lequel le domaine de signalisation est le domaine de signalisation d'immunoglobuline alpha ( $Ig\alpha$ ) décrit dans SEQ ID NO: 5, ou un variant de séquence de celui-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 5, ou dans lequel le domaine de signalisation est l'Ig membranaire décrite dans SEQ ID NO: 6, ou un variant de séquence de celle-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 6.

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2. Procédé selon la revendication 1, dans lequel l'échantillon est un échantillon d'air, un échantillon de liquide, un échantillon de légume, ou un échantillon sec, de préférence ledit échantillon est un échantillon biologique sélectionné dans le groupe consistant en du sang, du sérum, de la sueur, de l'urine, du liquide céphalorachidien, du mucus, du sperme, des selles, un liquide de lavage broncho-alvéolaire, et un tissu.

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3. Procédé selon la revendication 1, dans lequel l'agent est une toxine environnementale, un polluant, ou un médicament, de préférence ledit agent est un agent biologique sélectionné dans le groupe consistant en un agent de guerre biologique, un allergène, un antigène parasitaire, un antigène fongique, un antigène viral, un antigène bactérien, un antigène cellulaire, et un anticorps.

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4. Procédé selon la revendication 1, dans lequel la cellule de biocapteur est une cellule B, une cellule T, un monocyte, un macrophage, une cellule HEK293, une cellule CHO, une cellule P815, K562 ou Cos-1 qui exprime stablement la protéine de fusion chimérique et dans lequel l'activation cellulaire est une augmentation des taux de  $Ca^{2+}$  intracellulaire.
- 5
5. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel le domaine de liaison à un anticorps du  $Fc\gamma R$  est le domaine de liaison à un anticorps du  $Fc\gamma RI$  décrit dans SEQ ID NO:1 ou 3, ou un variant de séquence de celui-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 1 ou 3, ou dans lequel le domaine de liaison à un anticorps du  $Fc\gamma R$  est le domaine de liaison à un anticorps du  $Fc\gamma RIII$  décrit dans SEQ ID NO: 2 ou 4, ou un variant de séquence de celui-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 2 ou 4.
- 10
6. Cellule de biocapteur exprimant stablement une protéine de fusion chimérique, dans laquelle la protéine de fusion chimérique comprend un domaine de liaison à un anticorps du récepteur  $Fc\gamma$  ( $Fc\gamma R$ ) et un domaine de signalisation, et dans laquelle de préférence la cellule de biocapteur est une cellule B, une cellule T, un monocyte, un macrophage, une cellule HEK293, une cellule CHO, une cellule P815, K562 ou Cos-1 qui exprime stablement la protéine de fusion chimérique, dans laquelle le domaine de signalisation est le domaine de signalisation d'immunoglobuline alpha ( $Ig\alpha$ ) décrit dans SEQ ID NO: 5, ou un variant de séquence de celui-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 5, ou dans laquelle le domaine de signalisation est l'Ig membranaire décrite dans SEQ ID NO: 6, ou un variant de séquence de celle-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 6.
- 15
7. Cellule de biocapteur selon la revendication 6, dans laquelle le domaine de liaison à un anticorps du  $Fc\gamma R$  est le domaine de liaison à un anticorps du  $Fc\gamma RI$  décrit dans SEQ ID NO:1 ou 3, ou un variant de séquence de celui-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 1 ou 3, ou dans laquelle le domaine de liaison à un anticorps du  $Fc\gamma R$  est le domaine de liaison à un anticorps du  $Fc\gamma RIII$  décrit dans SEQ ID NO: 2 ou 4, ou un variant de séquence de celui-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 2 ou 4.
- 20
8. Protéine de fusion chimérique comprend un domaine de liaison à un anticorps du récepteur  $Fc\gamma$  ( $Fc\gamma R$ ) et un domaine de signalisation, dans laquelle le domaine de signalisation est le domaine de signalisation d'immunoglobuline alpha ( $Ig\alpha$ ) décrit dans SEQ ID NO: 5, ou un variant de séquence de celui-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 5, ou dans laquelle le domaine de signalisation est l'Ig membranaire décrite dans SEQ ID NO: 6, ou un variant de séquence de celle-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 6.
- 25
9. Protéine de fusion chimérique selon la revendication 8, dans laquelle le domaine de liaison à un anticorps du  $Fc\gamma R$  est le domaine de liaison à un anticorps du  $Fc\gamma RI$  décrit dans SEQ ID NO:1 ou 3, ou un variant de séquence de celui-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 1 ou 3, ou dans laquelle le domaine de liaison à un anticorps du  $Fc\gamma R$  est le domaine de liaison à un anticorps du  $Fc\gamma RIII$  décrit dans SEQ ID NO: 2 ou 4, ou un variant de séquence de celui-ci présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 2 ou 4.
- 30
10. Protéine de fusion chimérique selon la revendication 8, dans laquelle la protéine de fusion est la protéine de fusion  $Fc\gamma RI/Ig\alpha$  décrite dans SEQ ID NO: 8 ou un variant de séquence présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 8, ou dans laquelle la protéine de fusion est la protéine de fusion  $Fc\gamma RIII/Ig\alpha$  décrite dans SEQ ID NO:10 ou un variant de séquence présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 10.
- 35
11. Protéine de fusion chimérique selon la revendication 8, dans laquelle la protéine de fusion est la protéine de fusion  $Fc\gamma RI/Ig$  membranaire décrite dans SEQ ID NO: 22 ou un variant de séquence présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 22, ou dans laquelle la protéine de fusion est la protéine de fusion  $Fc\gamma RIII/Ig$  membranaire décrite dans SEQ ID NO: 23 ou un variant de séquence présentant au moins 95 % d'identité de séquence sur toute la longueur de SEQ ID NO: 23.
- 40
12. Séquence polynucléotidique codant pour une protéine de fusion chimérique selon l'une quelconque des revendications 8 à 11, ou un brin complémentaire de celle-ci.
- 45
- 50
- 55

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**13.** Vecteur de clonage ou cellule comprenant une séquence polynucléotidique selon la revendication 12.

**14.** Cellule comprenant un vecteur de clonage selon la revendication 13.

5 **15.** Procédé de production d'une protéine de fusion chimérique comprenant la mise en culture d'une cellule selon la revendication 13 ou d'une cellule selon la revendication 14, dans des conditions favorisant l'expression de la protéine de fusion, et la récupération de la protéine de fusion à partir de la cellule ou la culture cellulaire.

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Figure 1

**Construct**

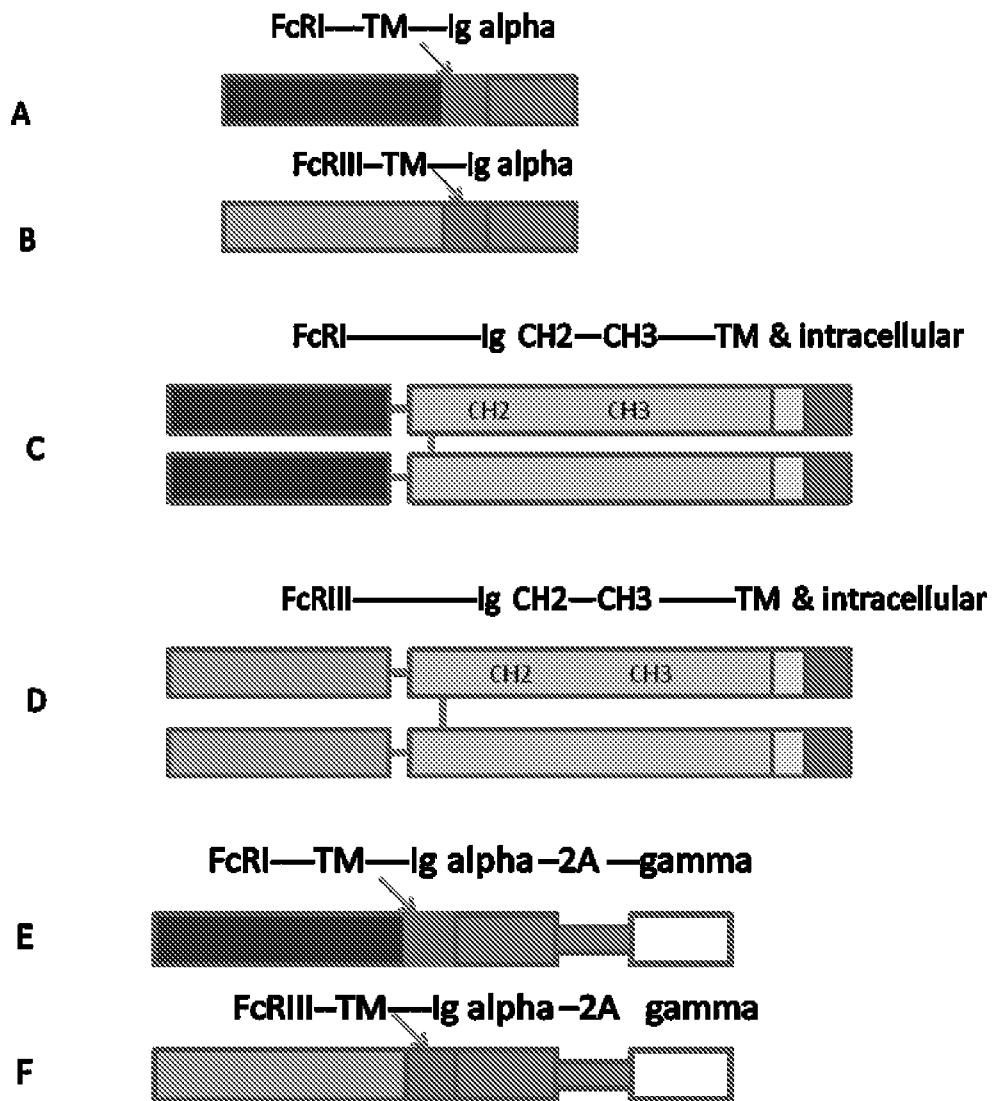


Figure 2

## Murine FcγRI

ATGATTCTTACCAGCTTTGGAGATGACATGTGGCTTCTAACAACTCTGCTACTT  
 M I L T S F G D D M W L L T T L L L  
 TGGGTTCCAGTCGGTGGGGAAGTGGTTAATGCCACCAAGGCTGTGATCACCTTGCAGCCT  
 W V P V G G E V V N A T K A V I T L Q P  
 CCATGGGTTCAGTATTTTCCAGAAGGAAAATGTCACCTTTATGGTGTGAGGGGCTCACCTG  
 P W V S I F Q K E N V T L W C E G P H L  
 CCTGGAGACAGTTCCACACAATGGTTTATCAACGGAACAGCCGTTTCAGATCTCCACGCCT  
 P G D S S T Q W F I N G T A V Q I S T P  
 AGTTATAGCATCCCAGAGGCCAGTTTTTCAGGACAGTGGCGAATACAGGTGTCAGATAGGT  
 S Y S I P E A S F Q D S G E Y R C Q I G  
 TCCTCAATGCCAAGTGACCCTGTGCAGTTGCAAATCCACAATGATTGGCTGCTACTCCAG  
 S S M P S D P V Q L Q I H N D W L L L Q  
 GCCTCCCGCAGAGTCCCTCACAGAAGGAGAACCCCTGGCCTTGAGGTGTCACGGATGGAAG  
 A S R R V L T E G E P L A L R C H G W K  
 AATAAACTGGTGTACAATGTGGTTTTCTATAGAAATGGAAAATCCTTTCAGTTTTCTTCA  
 N K L V Y N V V F Y R N G K S F Q F S S  
 GATTCGGAGGTCGCCATTCTGAAAACCAACCTGAGTCACAGCGGCATCTACCACTGCTCA  
 D S E V A I L K T N L S H S G I Y H C S  
 GGCACGGGAAGACACCGCTACACATCTGCAGGAGTGTCCATCACGGTGAAAGAGCTGTTT  
 G T G R H R Y T S A G V S I T V K E L F  
 ACCACGCCAGTGCTGAGAGCATCCGTGTCATCTCCCTTCCCGGAGGGGAGTCTGGTCACC  
 T T P V L R A S V S S P F P E G S L V T  
 CTGAACTGTGAGACGAATTTGCTCCTGCAGAGACCCGGCTTACAGCTTCACTTCTCCTTC  
 L N C E T N L L L Q R P G L Q L H F S F  
 TACGTGGGCAGCAAGATCCTGGAGTACAGGAACACATCCTCAGAGTACCATATAGCAAGG  
 Y V G S K I L E Y R N T S S E Y H I A R  
 GCGGAAAGAGAAGATGCTGGATTCTACTGGTGTGAGGTAGCCACGGAGGACAGCAGTGTG  
 A E R E D A G F Y W C E V A T E D S S V  
 CTTAAGCGCAGCCCTGAGTTGGAGCTCCAAGTGCTTGGTCCCCAGTCATCAGCTCCTGTC  
 L K R S P E L E L Q V L G P Q S S A P V  
 TGGTTTTACATCCTGTTTTATCTGTCAGTGGGAATAATGTTTTTCGTTGAACACGGTTCTC  
 W F H I L F Y L S V G I M F S L N T V L  
 TATGTGAAAATACACAGGCTGCAGAGAGAGAAGAAATACAACCTTAGAAGTCCCTTTGGTT  
 Y V K I H R L Q R E K K Y N L E V P L V  
 TCTGAGCAGGGAAAGAAAGCAAATTCCTTTCAGCAAGTTAGAAGCGATGGCGTGTATGAA  
 S E Q G K K A N S F Q Q V R S D G V Y E  
 GAAGTAACAGCCACTGCGAGCCAGACCACACAAAAGAAGCGCCGATGGACCTCGAAGC  
 E V T A T A S Q T T P K E A P D G P R S  
 TCAGTGGGTGACTGTGGACCCGAGCAGCCTGAACCCCTTCCCTCCAGTGACAGTACTGGG  
 S V G D C G P E Q P E P L P P S D S T G  
 GCACAACTTCCCAAAGTTGA  
 A Q T S Q S \*

Figure 3

## Murine FcγRIII

ATGACTTTGGACACCCAGATGTTTCAGAATGCACACTCTGGAAGCCAATGGCTACTT  
 M T L D T Q M F Q N A H S G S Q W L L  
 CCACCACTGACAATTCTGCTGCTGTTTGCAGACAGGCAGAGTGCAGCTCTTCCG  
 P P L T I L L L F A F A D R Q S A A L P  
 AAGGCTGTGGTGAAGACTGGACCCCCCATGGATCCAGGTGCTCAAGGAAGACATGGTGACA  
 K A V V K L D P P W I Q V L K E D M V T  
 CTGATGTGCGAAGGGACCCACAACCCTGGGAAGTCTTCTACTCAGTGGTTCACAAGTGG  
 L M C E G T H N P G N S S T Q W F H N W  
 AGTTCATCCGGAGCCAGGTCCAATCCAGCTACACGTTTAAGGCCACAGTCAATGACAGT  
 S S I R S Q V Q S S Y T F K A T V N D S  
 GGAGAATATCGGTGTCAAATGGAGCAGACCCGCCTCAGCGACCCTGTAGATCTGGGAGTG  
 G E Y R C Q M E Q T R L S D P V D L G V  
 ATTTCTGACTGGCTGCTGCTCCAGACCCCTCAGCGGGTGTCTTCTGGAAGGGAAACCATC  
 I S D W L L L Q T P Q R V F L E G E T I  
 ACGCTAAGGTGCCCTAGCTGGAGGAACAACTACTGAACAGGATCTCGTTCTTCCATAAT  
 T L R C P S W R N K L L N R I S F F H N  
 GAAAAATCCGTGAGGTATCATCACTACAAAAGTAATTTCTCTATCCCAAAGCCAACCAC  
 E K S V R Y H H Y K S N F S I P K A N H  
 AGTCACAGTGGGGACTACTACTGCAAAGGAAGTCTAGGAAGTACACAGCACCAGTCCAAG  
 S H S G D Y Y C K G S L G S T Q H Q S K  
 CCTGTCACCATCACTGTCCAAGACCCAGCAACTACATCCTCCATCTCTTAGTCTGGCAC  
 P V T I T V Q D P A T T S S I S L V W H  
 CACACTGCTTTCTCCCTAGTGATGTGCCTCCTGTTTGCAGTGGACACGGGCCTTTATTTT  
 H T A F S L V M C L L F A V D T G L Y F  
 TATGTACGGAGAAATCTTCAAACCCCGAGGGATTACTGGAGGAAGTCCCTGTCAATCAGA  
 Y V R R N L Q T P R D Y W R K S L S I R  
 AAGCACCAGGCTCCTCAAGACAAGTGA  
 K H Q A P Q D K \*

Figure 4

## Murine Iga

ATGCCAGGGGGTCTAGAAGCCCTCAGAGCCCTGCCTCTCCTCCTCTTCTTGTGCATACGCC  
 M P G G L E A L R A L P L L L F L S Y A  
 TGTTTGGGTCCCGGATGCCAGGCCCTGCGGGTAGAAGGGGGTCCACCATCCCTGACGGTG  
 C L G P G C Q A L R V E G G P P S L T V  
 AACTTGGGCGAGGAGGCCCGCCTCACCTGTGAAAACAATGGCAGGAACCCTAATATCACA  
 N L G E E A R L T C E N N G R N P N I T  
 TGGTGGTTCAGCCTTCAGTCTAACATCACATGGCCCCCAGTGCCACTGGGTCTGGCCAG  
 W W F S L Q S N I T W P P V P L G P G Q  
 GGTACCACAGGCCAGCTGTTCTTCCCCGAAGTAAACAAGAACCACAGGGGCTTGTACTGG  
 G T T G Q L F F P E V N K N H R G L Y W  
 TGCCAAGTGATAGAAAACAACATATTTAAAACGCTCCTGTGGTACTTACCTCCGCGTGCGC  
 C Q V I E N N I L K R S C G T Y L R V R  
 AATCCAGTCCCTAGGCCCTTCCCTGGACATGGGGGAAGGTACCAAGAACCGCATCATCACA  
 N P V P R P F L D M G E G T K N R I I T  
 GCAGAAGGGATCATCTTGCTGTTCTGTGCAGTGGTGCCAGGGACGCTGCTGCTATTCAGG  
 A E G I I L L F C A V V P G T L L L F R  
 AAACGGTGGCAAATGAGAAGTTTGGGGTGGACATGCCAGATGACTATGAAGATGAAAAT  
 K R W Q N E K F G V D M P D D Y E D E N  
 CTCTATGAGGGCCTGAACCTTGATGACTGTTCTATGTATGAGGACATCTCCAGGGGACTC  
 L Y E G L N L D D C S M Y E D I S R G L  
 CAGGGCACCTACCAGGATGTGGGCAACCTCCACATTGGAGATGCCCAGCTGGAAAAGCCA  
 Q G T Y Q D V G N L H I G D A Q L E K P  
 TGA

\*

Figure 5

## FcγRI/Igα fusion protein

ATGATTCTTACCAGCTTTGGAGATGACATGTGGCTTCTAACAACCTCTGCTACTT  
 M I L T S F G D D M W L L T T L L L  
 TGGGTTCCAGTCGGTGGGGAAGTGGTTAATGCCACCAAGGCTGTGATCACCTTGCAGCCT  
 W V P V G G E V V N A T K A V I T L Q P  
 CCATGGGTCAGTATTTTCCAGAAGGAAAATGTCACCTTATGGTGTGAGGGGCCTCACCTG  
 P W V S I F Q K E N V T L W C E G P H L  
 CCTGGAGACAGTTCCACACAATGGTTTATCAACGGAACAGCCGTTTCCAGATCTCCACGCCT  
 P G D S S T Q W F I N G T A V Q I S T P  
 AGTTATAGCATCCCAGAGGCCAGTTTTTCCAGGACAGTGGCGAATACAGGTGTCAGATAGGT  
 S Y S I P E A S F Q D S G E Y R C Q I G  
 TCCTCAATGCCAAGTGACCCTGTGCAGTTGCAAATCCACAATGATTGGCTGCTACTCCAG  
 S S M P S D P V Q L Q I H N D W L L L Q  
 GCCTCCCGCAGAGTCCACAGAAGGAGAACCCCTGGCCTTGAGGTGTCACGGATGGAAG  
 A S R R V L T E G E P L A L R C H G W K  
 AATAAACTGGTGTACAATGTGGTTTTTCTATAGAAATGGAAAATCCTTTTCCAGTTTTTCTTCA  
 N K L V Y N V V F Y R N G K S F Q F S S  
 GATTTCGGAGGTCGCCATTCTGAAAACCAACCTGAGTCACAGCGGCATCTACCACTGCTCA  
 D S E V A I L K T N L S H S G I Y H C S  
 GGCACGGGAAGACACCGCTACACATCTGCAGGAGTGTCCATCACGGTGAAAGAGCTGTTT  
 G T G R H R Y T S A G V S I T V K E L F  
 ACCACGCCAGTGTGAGAGCATCCGTGTCATCTCCCTTCCCGGAGGGGAGTCTGGTCACC  
 T T P V L R A S V S S P F P E G S L V T  
 CTGAACTGTGAGACGAATTTGCTCCTGCAGAGACCCGGCTTACAGCTTCACTTCTCCTTC  
 L N C E T N L L L Q R P G L Q L H F S F  
 TACGTGGGCAGCAAGATCCTGGAGTACAGGAACACATCCTCAGAGTACCATATAGCAAGG  
 Y V G S K I L E Y R N T S S E Y H I A R  
 GCGGAAAGAGAAGATGCTGGATTCTACTGGTGTGAGGTAGCCACGGAGGACAGCAGTGTC  
 A E R E D A G F Y W C E V A T E D S S V  
 CTTAAGCGCAGCCCTGAGTTGGAGCTCCAAGTGCTTGGTCCCCAGTCATCAGCTCCTGTC  
 L K R S P E L E L Q V L G P Q S S A P V  
 TGGTTTCCACATCCTGTTTTATCTGTCAGTGGGAATAATGTTTTTCGTTGAACACGGTTCTC  
 W F H I L F Y L S V G I M F S L N T V L  
 TATGTGTTTCCAGGAAACGGTGGCAAATGAGAAGTTTGGGGTGGACATGCCAGATGACTAT  
 Y V F R K R W Q N E K F G V D M P D D Y  
 GAAGATGAAAATCTCTATGAGGGCCTGAACCTTGATGACTGTTCTATGTATGAGGACATC  
 E D E N L Y E G L N L D D C S M Y E D I  
 TCCAGGGGACTCCAGGGCACCTACCAGGATGTGGGCAACCTCCACATTGGAGATGCCAG  
 S R G L Q G T Y Q D V G N L H I G D A Q  
 CTGGAAAAGCCATGA  
 L E K P \*

Figure 6

Fc $\gamma$ RIII/Ig $\alpha$  fusion protein

ATGACTTTGGACACCCAGATGTTTCAGAATGCACACTCTGGAAGCCAATGGCTACTT  
 M T L D T Q M F Q N A H S G S Q W L L  
 CCACCACTGACAATTCTGCTGCTGTTTGCAGACAGGCAGAGTGCAGCTCTTCCG  
 P P L T I L L L F A F A D R Q S A A L P  
 AAGGCTGTGGTGAAGTGGACCCCATGGATCCAGGTGCTCAAGGAAGACATGGTGACA  
 K A V V K L D P P W I Q V L K E D M V T  
 CTGATGTGCGAAGGGACCCACAACCCTGGGAAGTCTTCTACTCAGTGGTTCCACAAGTGG  
 L M C E G T H N P G N S S T Q W F H N W  
 AGTTCATCCGGAGCCAGGTCCAATCCAGCTACACGTTTAAGGCCACAGTCAATGACAGT  
 S S I R S Q V Q S S Y T F K A T V N D S  
 GGAGAATATCGGTGTCAAATGGAGCAGACCCGCCTCAGCGACCCTGTAGATCTGGGAGTG  
 G E Y R C Q M E Q T R L S D P V D L G V  
 ATTTCTGACTGGCTGCTGCTCCAGACCCCTCAGCGGGTGTCTTCTGGAAGGGGAAACCATC  
 I S D W L L L Q T P Q R V F L E G E T I  
 ACGCTAAGGTGCCCTAGCTGGAGGAACAACTACTGAACAGGATCTCGTTCTTCCATAAT  
 T L R C P S W R N K L L N R I S F F H N  
 GAAAAATCCGTGAGGTATCATCACTACAAAAGTAATTTCTCTATCCCAAAGCCAACCAC  
 E K S V R Y H H Y K S N F S I P K A N H  
 AGTCACAGTGGGGACTACTACTGCAAAGGAAGTCTAGGAAGTACACAGCACCAGTCCAAG  
 S H S G D Y Y C K G S L G S T Q H Q S K  
 CCTGTACCATCACTGTCCAAGACCCAGCAACTACATCCTCCATCTCTCTAGTCTGGCAC  
 P V T I T V Q D P A T T S S I S L V W H  
 CACTGCTTTCTCCCTAGTGATGTGCCTCCTGTTTGCAGTGTTCAGGAAACGGTGGCAA  
 H T A F S L V M C L L F A V F R K R W Q  
 AATGAGAAGTTTGGGGTGGACATGCCAGATGACTATGAAGATGAAAATCTCTATGAGGGC  
 N E K F G V D M P D D Y E D E N L Y E G  
 CTGAACCTTGATGACTGTTCTATGTATGAGGACATCTCCAGGGGACTCCAGGGCACCTAC  
 L N L D D C S M Y E D I S R G L Q G T Y  
 CAGGATGTGGGCAACCTCCACATTGGAGATGCCAGCTGGAAAAGCCATGA  
 Q D V G N L H I G D A Q L E K P \*

Figure 7

## Membrane Ig

GAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCA  
 E R K C C V E C P P C P A P P V A G P S  
 GTCTTCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCCTGAGGTC  
 V F L F P P K P K D T L M I S R T P E V  
 ACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTG  
 T C V V V D V S H E D P E V Q F N W Y V  
 GACGGCATGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACG  
 D G M E V H N A K T K P R E E Q F N S T  
 TTCCGTGTGGTCAGCGTCCTCACCGTCGTGCACCAGGACTGGCTGAACGGCAAGGAGTAC  
 F R V V S V L T V V H Q D W L N G K E Y  
 AAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAACC  
 K C K V S N K G L P A P I E K T I S K T  
 AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC  
 K G Q P R E P Q V Y T L P P S R E E M T  
 AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTG  
 K N Q V S L T C L V K G F Y P S D I A V  
 GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACACCTCCCATGCTGGAC  
 E W E S N G Q P E N N Y K T T P P M L D  
 TCCGACGGCTCCTTCTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAG  
 S D G S F F L Y S K L T V D K S R W Q Q  
 GGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAG  
 G N V F S C S V M H E A L H N H Y T Q K  
 AGCCTCTCCCTGTCTCCGGAGCTGCAACTGGAGGAGAGCTGTGCGGAGGCGCAGGACGGG  
 S L S L S P E L Q L E E S C A E A Q D G  
 GAGCTGGACGGGCTGTGGACGACCATCACCATCTTCATCACACTCTTCCTGCTAAGCGTG  
 E L D G L W T T I T I F I T L F L L S V  
 TGCTACAGTGCCACCATCACCTTCTTCAAGGTGAAGTGGATCTTCTCCTCAGTGGTGGAC  
 C Y S A T I T F F K V K W I F S S V V D  
 CTGAAGCAGACCATCGTCCCCGACTACAGGAACATGATCAGGCAGGGGGCCTAG  
 L K Q T I V P D Y R N M I R Q G A \*

Figure 8/1

Fc $\gamma$ RI/membrane Ig fusion protein

ATGATTCTTACCAGCTTTGGAGATGACATGTGGCTTCTAACAACTCTGCTACTT  
 M I L T S F G D D M W L L T T L L L  
 TGGGTTCCAGTCGGTGGGGAAGTGGTTAATGCCACCAAGGCTGTGATCACCTTGCAGCCT  
 W V P V G G E V V N A T K A V I T L Q P  
 CCATGGGTCAAGTATTTTCCAGAAGGAAAATGTCACCTTTATGGTGTGAGGGGCTCACCTG  
 P W V S I F Q K E N V T L W C E G P H L  
 CCTGGAGACAGTTCACACAATGGTTTATCAACGGAACAGCCGTTTCCAGATCTCCACGCCT  
 P G D S S T Q W F I N G T A V Q I S T P  
 AGTTATAGCATCCCAGAGGCCAGTTTTTTCAGGACAGTGGCGAATACAGGTGTCAGATAGGT  
 S Y S I P E A S F Q D S G E Y R C Q I G  
 TCCTCAATGCCAAGTGACCCTGTGCAGTTGCAAATCCACAATGATTGGCTGCTACTCCAG  
 S S M P S D P V Q L Q I H N D W L L L Q  
 GCCTCCCGCAGAGTCCCTCACAGAAGGAGAACCCCTGGCCTTGAGGTGTCACGGATGGAAG  
 A S R R V L T E G E P L A L R C H G W K  
 AATAAACTGGTGTACAATGTGGTTTTTCTATAGAAATGGAAAATCCTTTCAGTTTTTCTTCA  
 N K L V Y N V V F Y R N G K S F Q F S S  
 GATTCGGAGGTCGCCATTCTGAAAACCAACCTGAGTCACAGCGGCATCTACCACTGCTCA  
 D S E V A I L K T N L S H S G I Y H C S  
 GGCACGGGAAGACACCGCTACACATCTGCAGGAGTGTCCATCACGGTGAAAGAGCTGTTT  
 G T G R H R Y T S A G V S I T V K E L F  
 ACCACGCCAGTGCTGAGAGCATCCGTGTCATCTCCCTTCCCGGAGGGGAGTCTGGTCACC  
 T T P V L R A S V S S P F P E G S L V T  
 CTGAACTGTGAGACGAATTTGCTCCTGCAGAGACCCGGCTTACAGCTTCACTTCTCCTTC  
 L N C E T N L L L Q R P G L Q L H F S F  
 TACGTGGGCAGCAAGATCCTGGAGTACAGGAACACATCCTCAGAGTACCATATAGCAAGG  
 Y V G S K I L E Y R N T S S E Y H I A R  
 GCGGAAAGAGAAGATGCTGGATTCTACTGGTGTGAGGTAGCCACGGAGGACAGCAGTGTG  
 A E R E D A G F Y W C E V A T E D S S V  
 CTTAAGCGCAGCCCTGAGTTGGAGGAGCGCAAATGTTGTGTGCGAGTGCCACCGTGCCCA  
 L K R S P E L E E R K C C V E C P P C P  
 GCACCACCTGTGGCAGGACCGTCAGTCACCCCTCATGATCTCCCGGACCCCTGAGGTACCG  
A P P V A G P S V T L M I S R T P E V T  
TTCTCTTCCCCCAAACCAAGGACGACCCCGAGGTCCAGTTCAACTGGTACGTGGAC  
F L F P P K P K D D P E V Q F N W Y V D  
TGCGTGGTGGTGGACGTGAGCCACGAAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTT  
C V V V D V S H E K P R E E Q F N S T F  
GGCATGGAGGTGCATAATGCCAAGACACACCAGGACTGGCTGAACGGCAAGGAGTACAAG  
G M E V H N A K T H Q D W L N G K E Y K  
CGTGTGGTCAAGTCCCTCACCGTTCGTGGCCCCATCGAGAAAACCATCTCCAAAACCAAA  
R V V S V L T V V A P I E K T I S K T K  
TGCAAGGTCTCCAACAAGGCCTCCCAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG  
C K V S N K G L P G Q P R E P Q V Y T L

Figure 8/2

CCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC  
P P S R E E M T K N Q V S L T C L V K G  
TTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTAC  
F Y P S D I A V E W E S N G Q P E N N Y  
AAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC  
K T T P P M L D S D G S F F L Y S K L T  
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT  
V D K S R W Q Q G N V F S C S V M H E A  
CTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGAGCTGCAACTGGAGGAG  
L H N H Y T Q K S L S L S P E L Q L E E  
AGCTGTGCGGAGGCGCAGGACGGGGAGCTGGACGGGCTGTGGACGACCATCACCATCTTC  
S C A E A Q D G E L D G L W T T I T I F  
ATCACACTCTTCCTGCTAAGCGTGTGCTACAGTGCCACCATCACCTTCTTCAAGGTGAAG  
I T L F L L S V C Y S A T I T F F K V K  
TGGATCTTCTCCTCAGTGGTGGACCTGAAGCAGACCATCGTCCCCGACTACAGGAACATG  
W I F S S V V D L K Q T I V P D Y R N M  
ATCAGGCAGGGGGCCTAG  
I R Q G A

Figure 9/1

Fc $\gamma$ R1III/membrane Ig fusion protein

ATGACTTTGGACACCCAGATGTTTCAGAATGCACACTCTGGAAGCCAATGGCTACTT  
 M T L D T Q M F Q N A H S G S Q W L L  
 CCACCACTGACAATTCTGCTGCTGTTTGGCTTTTGCAGACAGGCAGAGTGCAGCTCTTCCG  
 P P L T I L L L F A F A D R Q S A A L P  
 AAGGCTGTGGTGGAACTGGACCCCCCATGGATCCAGGTGCTCAAGGAAGACATGGTGACA  
 K A V V K L D P P W I Q V L K E D M V T  
 CTGATGTGCGAAGGGACCCACAACCCTGGGAACTCTTCTACTCAGTGGTTCCACAACCTGG  
 L M C E G T H N P G N S S T Q W F H N W  
 AGTTCCATCCGGAGCCAGGTCCAATCCAGCTACACGTTTAAGGCCACAGTCAATGACAGT  
 S S I R S Q V Q S S Y T F K A T V N D S  
 GGAGAATATCGGTGTCAAATGGAGCAGACCCGCCTCAGCGACCCTGTAGATCTGGGAGTG  
 G E Y R C Q M E Q T R L S D P V D L G V  
 ATTTCTGACTGGCTGCTGCTCCAGACCCCTCAGCGGGTGTTCCTGGAAGGGGAAACCATC  
 I S D W L L L Q T P Q R V F L E G E T I  
 ACGCTAAGGTGCCCTAGCTGGAGGAACAACTACTGAACAGGATCTCGTTCTTCCATAAT  
 T L R C P S W R N K L L N R I S F F H N  
 GAAAAATCCGTGAGGTATCATCACTACAAAAGTAATTTCTCTATCCCAAAGCCAACCAC  
 E K S V R Y H H Y K S N F S I P K A N H  
 AGTCACAGTGGGGACTACTACTGCAAAGGAAGTCTAGGAAGTACACAGCACCAGTCCAAG  
 S H S G D Y Y C K G S L G S T Q H Q S K  
 CCTGTCACCATCACTGTCCAAGACGAGCGCAAATGTTGTGTCGAGTGCCACCCGTGCCCA  
 P V T I T V Q D E R K C C V E C P P C P  
 GCACCACCTGTGGCAGGACCGTCAGTCTTCTCTTCCCCC~~AAAACCC~~AAGGACACCCCTC  
A P P V A G P S V F L F P P K P K D T L  
 ATGATCTCCCGGACCCCTGAGGTACAGTGCCTGGTGGTGGACGTGAGCCACGAAGACCCC  
M I S R T P E V T C V V V D V S H E D P  
 GAGGTCCAGTTCAACTGGTACGTGGACGGCATGGAGGTGCATAATGCCAAGACAAAGCCA  
E V Q F N W Y V D G M E V H N A K T K P  
 CGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTCGTGCACCAG  
R E E Q F N S T F R V V S V L T V V H Q  
 GACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTCTCCAACAAAGGCCTCCAGCCCCC  
D W L N G K E Y K C K V S N K G L P A P  
 ATCGAGAAAACCATCTCCAAAACCAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG  
I E K T I S K T K G Q P R E P Q V Y T L  
 CCCCCATCCCGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGC  
P P S R E E M T K N Q V S L T C L V K G  
 TTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACAACACTAC  
F Y P S D I A V E W E S N G Q P E N N Y  
 AAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACC  
K T T P P M L D S D G S F F L Y S K L T  
 GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT  
V D K S R W Q Q G N V F S C S V M H E A

Figure 9/2

CTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGAGCTGCAACTGGAGGAG  
L H N H Y T Q K S L S L S P E L Q L E E  
AGCTGTGCGGAGGGCGCAGGACGGGGAGCTGGACGGGCTGTGGACGACCATCACCATCTTC  
S C A E A Q D G E L D G L W T T I T I F  
ATCACACTCTTCCTGCTAAGCGTGTGCTACAGTGCCACCATCACCTTCTTCAAGGTGAAG  
I T L F L L S V C Y S A T I T F F K V K  
TGGATCTTCTCCTCAGTGGTGGACCTGAAGCAGACCATCGTCCCCGACTACAGGAACATG  
W I F S S V V D L K Q T I V P D Y R N M  
ATCAGGCAGGGGCCTAG  
I R Q G A

Figure 10

2A Sequence

GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCGGAGACGGGAGGAGAACCCTGG  
G S G A T N F S L L K Q A E T G G E P W  
ACC  
T

Figure 11

Mouse FcR gamma chain

ATGATCTCAGCCGTGATCTTGTTCTTGCTCCTT  
M I S A V I L F L L L  
TTGGTGGAACAAGCAGCCGCCCTGGGAGAGCCGCAGCTCTGCTATATCCTGGATGCTGTC  
L V E Q A A A L G E P Q L C Y I L D A V  
CTGTTTTTGTATGGTATTGTCCTTACCCTACTCTACTGTCGACTCAAGATCCAGGTCCGA  
L F L Y G I V L T L L Y C R L K I Q V R  
AAGGCAGCTATAGCCAGCCGTGAGAAAGCAGATGCTGTCTACACGGGCCTGAACACCCGG  
K A A I A S R E K A D A V Y T G L N T R  
AGCCAGGAGACATATGAGACTCTGAAGCATGAGAAACCACCCAGTAG  
S Q E T Y E T L K H E K P P Q \*

## REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	通用抗体介导的生物传感器		
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外部链接	<a href="#">Espacenet</a>		

摘要(译)

提供了一种通用抗体介导的生物传感器，其包括稳定表达可用于检测样品中靶标试剂的新型嵌合融合蛋白的生物传感器细胞系。融合蛋白具有结合抗体而不考虑其结合特异性的细胞外抗体结合结构域和在抗原结合时诱导细胞活化的信号传导结构域。由于融合蛋白与任何抗体的Fc区结合，因此它可以充当细胞外信号传导和细胞内激活之间的通用途径。生物传感器可用于检测样品中所选抗原的存在。

Met Ile Leu Thr Ser Phe Gly Asp Asp Met Trp Leu Leu Thr Thr Leu  
1 5 10 15  
Leu Leu Trp Val Pro Val Gly Gly Glu Val Val Asn Ala Thr Lys Ala  
20 25 30  
Val Ile Thr Leu Gln Pro Pro Trp Val Ser Ile Phe Gln Lys Glu Asn  
35 40 45  
Val Thr Leu Trp Cys Glu Gly Pro His Leu Pro Gly Asp Ser Ser Thr  
50 55 60  
Gln Trp Phe Ile Asn Gly Thr Ala Val Gln Ile Ser Thr Pro Ser Tyr  
65 70 75 80  
Ser Ile Pro Glu Ala Ser Phe Gln Asp Ser Gly Glu Tyr Arg Cys Gln  
85 90 95  
Ile Gly Ser Ser Met Pro Ser Asp Pro Val Gln Leu Gln Ile His Asn  
100 105 110  
Asp Trp Leu Leu Leu Gln Ala Ser Arg Arg Val Leu Thr Glu Gly Glu  
115 120 125  
Pro Leu Ala Leu Arg Cys His Gly Trp Lys Asn Lys Leu Val Tyr Asn  
130 135 140  
Val Val Phe Tyr Arg Asn Gly Lys Ser Phe Gln Phe Ser Ser Asp Ser  
145 150 155 160  
Glu Val Ala Ile Leu Lys Thr Asn Leu Ser His Ser Gly Ile Tyr His