US 20070184501A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2007/0184501 A1**

Odegaard (43) **Pub. Date: Aug. 9, 2007**

(54) **BLOCKER REAGENT FOR REDUCTION OF HETEROPHILE INTERFERENCES**

(75) Inventor: **Bruce H. Odegaard**, Crystal, MN (US)

Correspondence Address:
BECKMAN COULTER, INC.
P.O. BOX 169015
MAIL CODE 32-A02
MIAMI, FL 33116-9015 (US)

(73) Assignee: **Beckman Coulter, Inc.**, Fullerton, CA

(21) Appl. No.: **11/349,444**

(22) Filed: **Feb. 7, 2006**

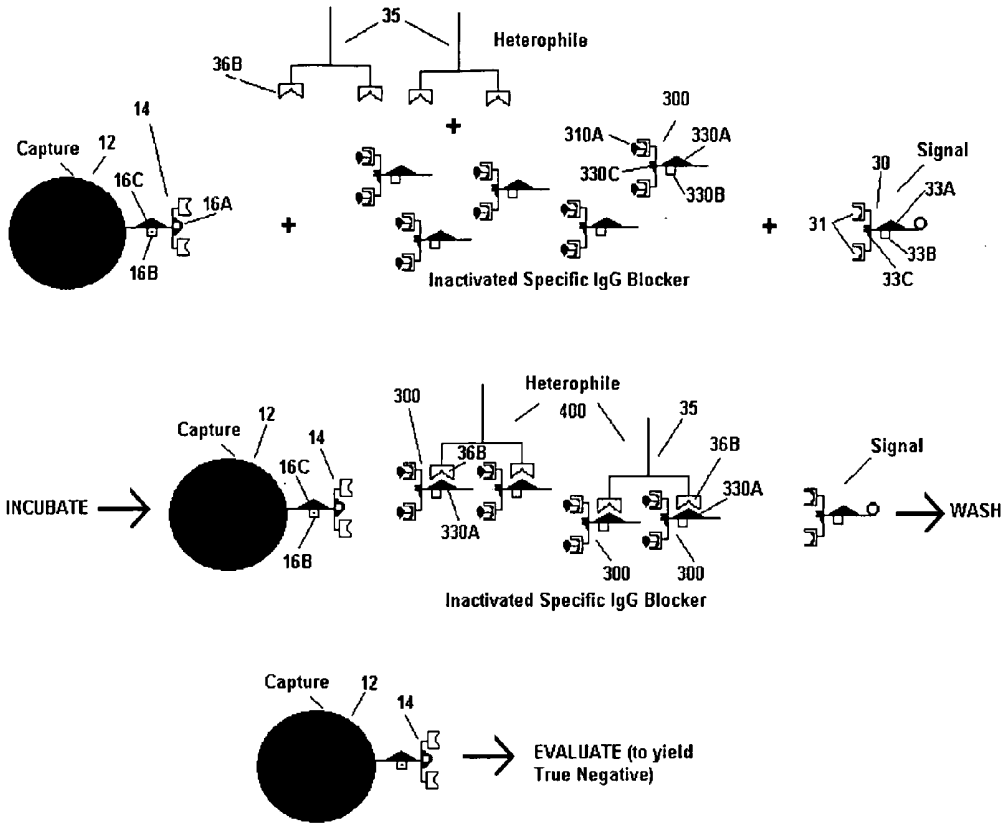
Publication Classification

(51) **Int. Cl.**
G01N 33/53 (2006.01)

(52) **U.S. Cl.** **435/7.92**

(57) **ABSTRACT**

A specific blocking reagent, method of manufacture thereof and methods of use are provided. The blocking reagent uses the specific antibody structure recognized by the target, e.g., analyte, and any interfering substances, e.g., heterophile molecules. The blocking reagent is, however, an inactivated form of the target-specific antibody. Thus, the blocking reagent cannot bind the target because the target-binding regions have been inactivated. The blocking reagent can, however, block interfering substances such as heterophile molecules as these binding regions remain active. In this manner, the blocking reagent provides highly specific removal of interferents. The blocking reagent thus has broad applicability in immunoassays as well as enhanced delivery of therapeutic and/or diagnostic agents.



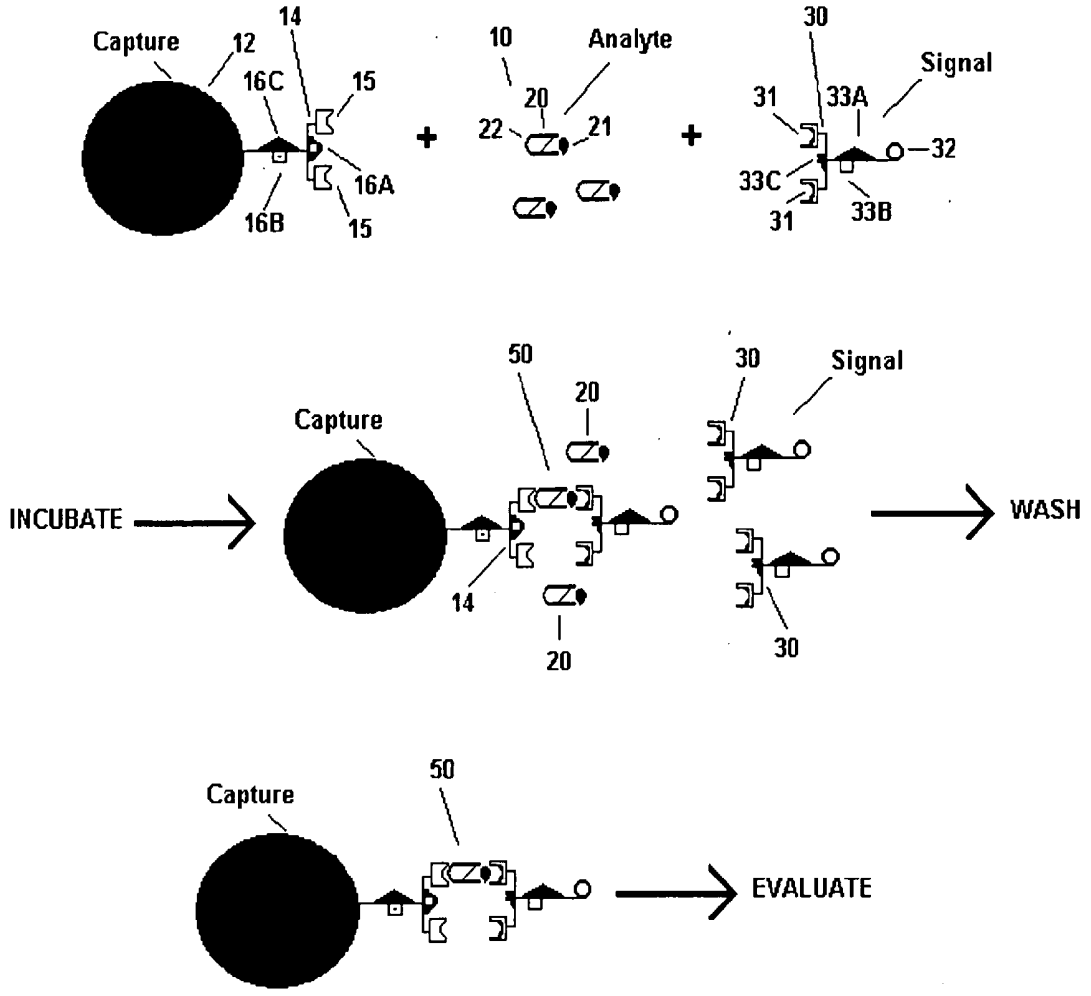
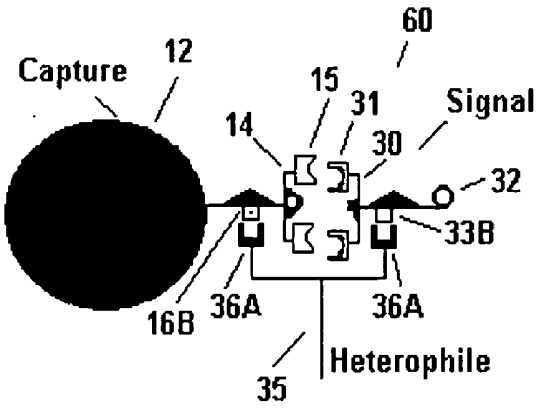
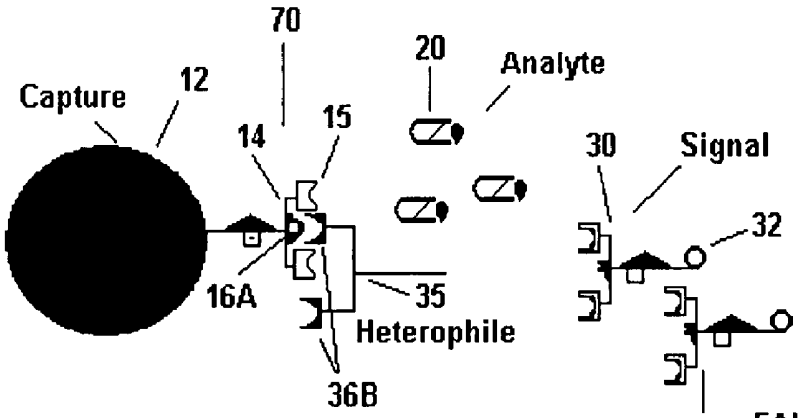


Fig. 1
Prior Art



FALSE POSITIVE
(signal without
analyte)

Fig. 2A
Prior Art



FALSE NEGATIVE
(no signal with
analyte)

Fig. 2B
Prior Art

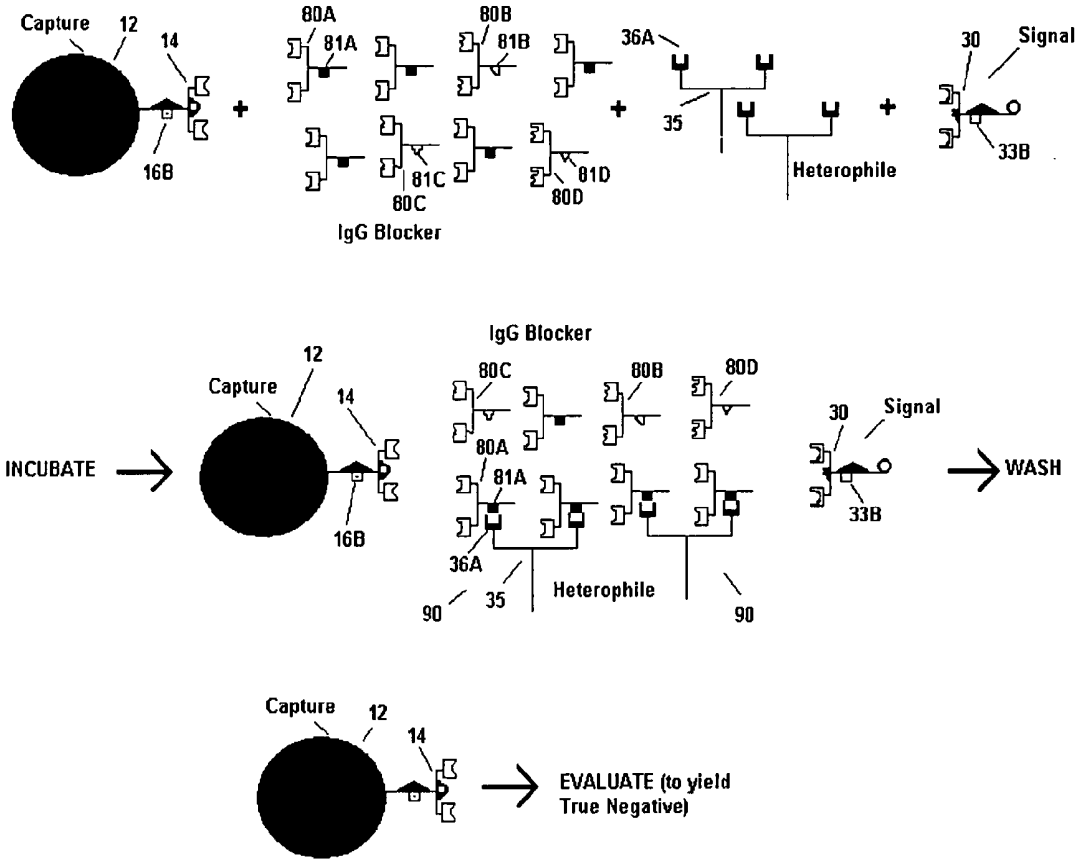


Fig. 3
Prior Art

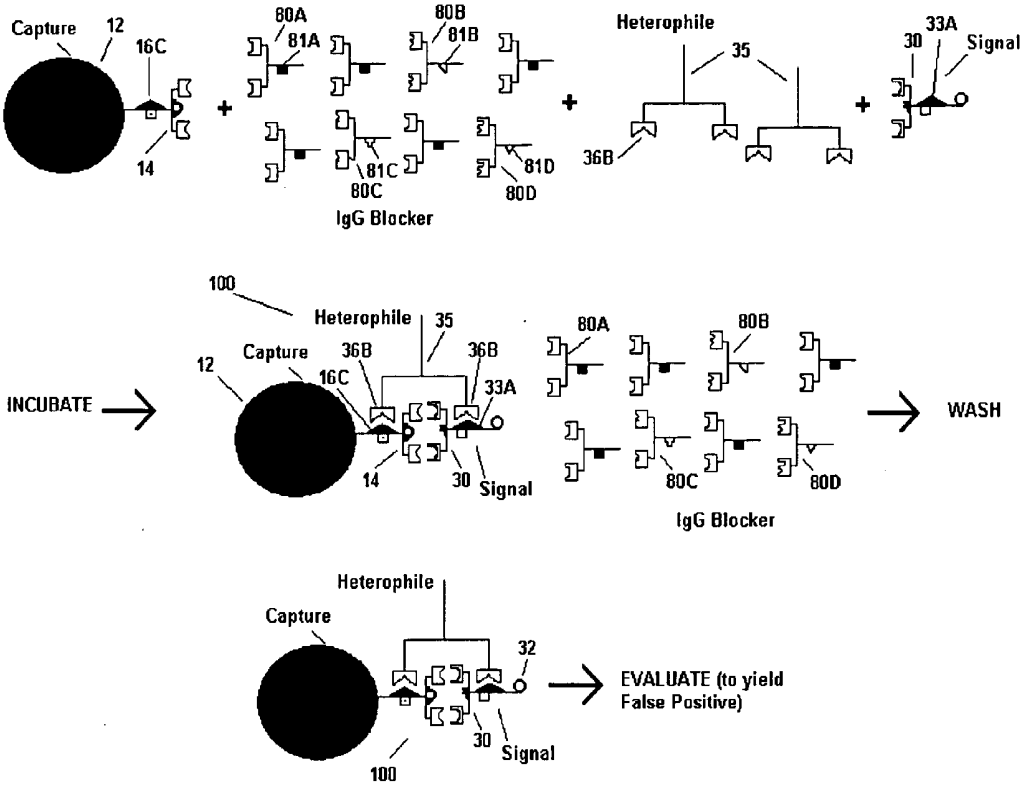


Fig. 4
Prior Art

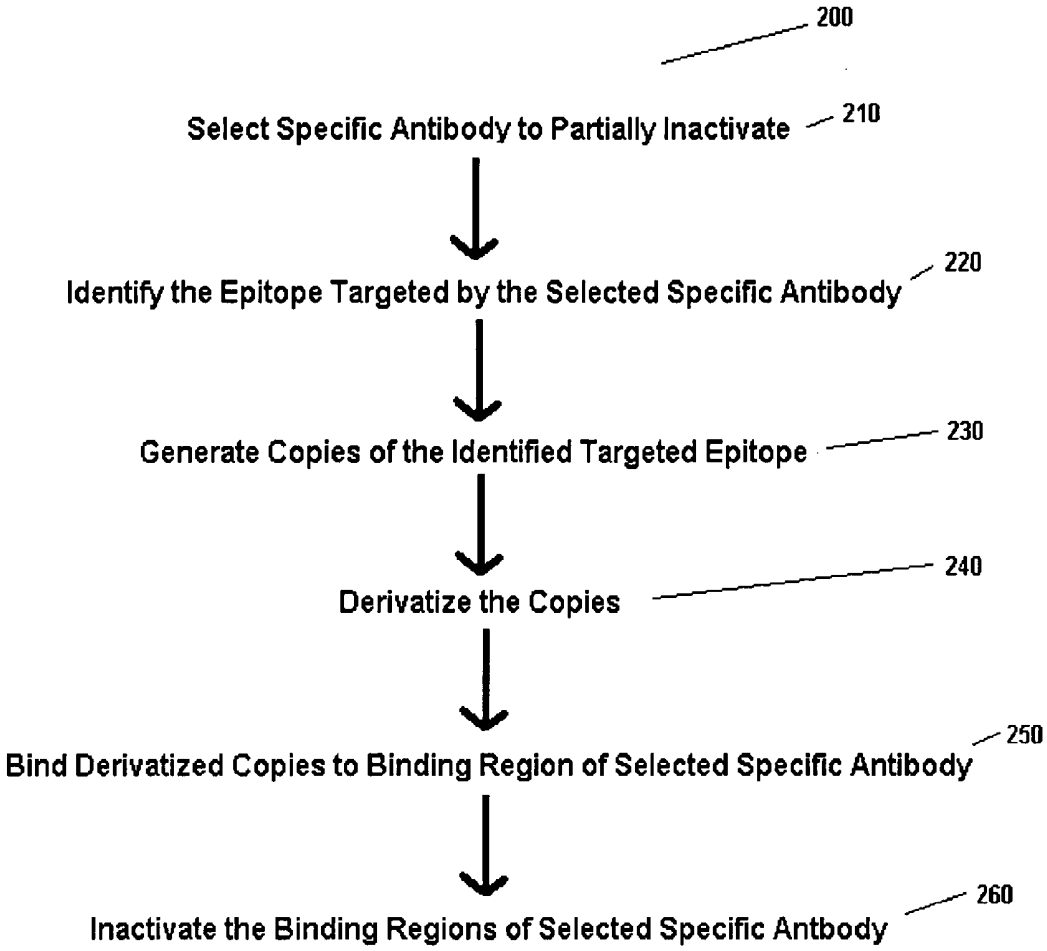

200
Select Specific Antibody to Partially Inactivate
210
Identify the Epitope Targeted by the Selected Specific Antibody
220
Generate Copies of the Identified Targeted Epitope
230
Derivatize the Copies
240
Bind Derivatized Copies to Binding Region of Selected Specific Antibody
250
Inactivate the Binding Regions of Selected Specific Antbody
260

Fig. 5

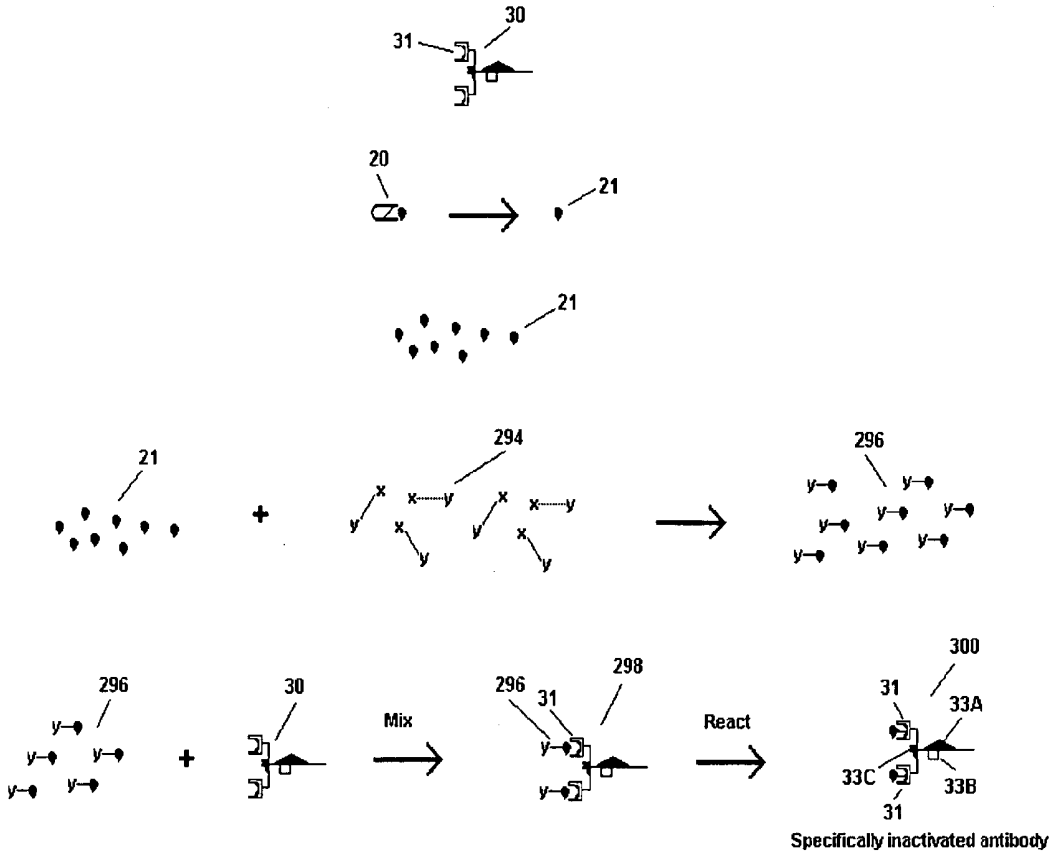

30
31
20
21
21
21
294
296
296
30
Mix
296
31
298
React
300
31
33A
33C
33B
31
Specifically inactivated antibody

Fig. 6

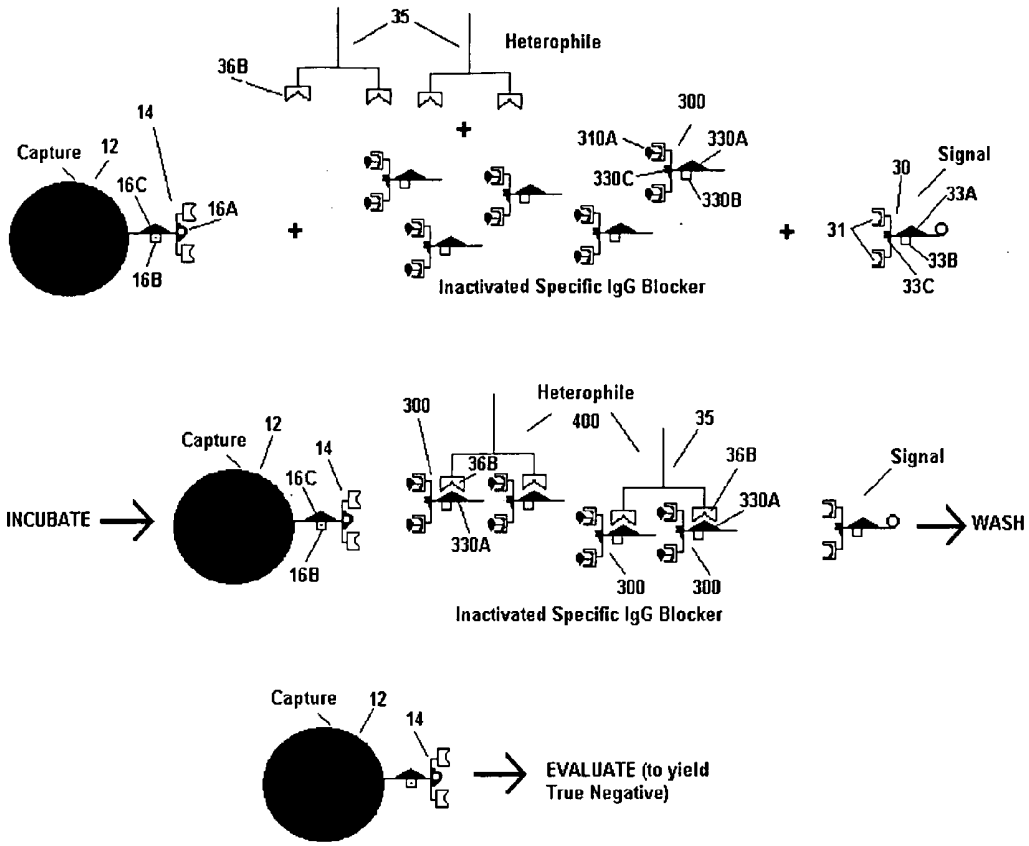


Fig. 7 The Invention

BLOCKER REAGENT FOR REDUCTION OF HETEROPHILE INTERFERENCES

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] The present invention relates generally to reagent, method of manufacture and method of use for specifically reducing interference by a substance with a target. More specifically, reagents and methods are provided that utilize substantially identical epitopic structures recognized by a particular interferent, e.g., heterophiles, that are incapable of binding the analyte of interest to obtain analyte-specific reduction of heterophile interference in immunoassays. The reagent and methods also are applicable in improving the specific delivery of diagnostic and/or therapeutic agents.

[0003] 2. Description of the Related Art

[0004] Immunoassays are assay reagent systems that exploit the ability of an antibody to specifically recognize and bind to a particular analyte or "antigen." An antigen is a substance which is capable of inducing an immune response, i.e., antibody production, when introduced into an animal or human body. The particular region of an antigen or analyte that is recognized by an antibody, and to which the antibody binds, is referred to as an "epitope" or binding region.

[0005] The simplest immunoassay reagent system involves merely incubating an antibody that is capable of binding to a target molecule (i.e., the "analyte") with a sample that is suspected to contain the analyte. The presence of the target molecule is determined by the presence, and is proportional to the concentration, of any immune complexes that form through the binding of the antibody and the analyte. In order to facilitate the separation of such immune complexes from uncomplexed material, a solid phase is typically employed. In more sophisticated immunoassays such as particle-enhanced assays, the concentration of the target molecule is determined by binding the antibody to a support such as latex particles, and then incubating the support-bound antibody in the presence of the analyte-containing sample.

[0006] Target molecules that have become bound to the immobilized antibody can be detected in a variety of ways. For example, the support can be incubated in the presence of a labeled, second antibody (i.e., a "sandwich" immunoassay) that is capable of binding to a second epitope of the target molecule. Binding of the labeled antibody on the support thus requires the presence of the target, and is proportional to the concentration of the target in the sample.

[0007] Alternatively, the sample is incubated with a known amount of labeled target molecule and antibody binding sites. Any target molecules in the sample compete with the labeled target molecules for the antibody binding sites. Thus, the amount of labeled target molecules that are able to bind the antibody is inversely proportional to the concentration of target molecules in the sample. This is known as a competitive immunoassay.

[0008] The various immunoassay formats can be further divided into two main classes, depending upon whether the assay requires the separation of bound species from unbound species. Heterogeneous immunoassays require such separation, and hence entail a separation or isolation step. In contrast, homogeneous assays are designed such that the removal of bound species from unbound species is unnecessary. Because homogeneous assays lack a separation step, and are more easily automated, they are more desirable than heterogeneous assays in applications that require the screening of large numbers of patients.

[0009] In particle-enhanced immunoassays, an immune complex formation caused by a reaction between one or more particle-bound antibodies and the analyte results in particle aggregation. If the immune complex is large enough, it will become capable of scattering light, or of spontaneously precipitating. In such cases, agglutination, nephelometric, or turbidimetric detection methods may be employed. Nephelometric methods measure the light scattered by a suspension of particles or reflected toward a detector that is not in the direct path of light. In contrast, turbidimetric methods measure the reduction of light transmitted through the suspension of particles or aggregates. The reduction is caused by reflection, scatter, and absorption of the light by the aggregates. Agglutination assays measure the precipitation of antibody-antigen complexes. Such assays can be extremely sensitive, and are amenable to automation. Because nephelometric and turbidimetric methods do not require the separation of the initially present antibody from the immune complexes formed in the assay, such assays are homogenous immunoassays.

[0010] Heterophile molecules are a well-known cause of interference in all immunoassay types and formats and are present in up to 40% of normal blood donors. Heterophile molecules are produced against poorly defined antigens and generally show weak avidity and may be multispecies specific. Heterophiles may be IgAs, IgEs, IgGs, IgMs or Ig(n) and may be poly- or mono-specific.

[0011] Heterophile antibodies may interfere in two-site immunoassays by causing false-positive results. This form of interference results from the heterophile molecule bridging the capture and signal antibodies, in the presence or absence of the target analyte, yielding a false-positive result. Alternatively, heterophile molecules may cause false-negative results by binding directly with the capture antibody, thereby blocking the capture antibody's reactive site from binding the target analyte. Either type of interference results in decreased sensitivity or specificity of the affected immunoassay.

[0012] Current approaches to reducing heterophilic interference in immunoassays focus on flooding the patient sample and/or subsequent immunochemical reaction mixture with a broad range of non-specific "normal IgG" antibodies, or in some cases monoclonal IgG antibody of the same isotype. These approaches simply rely on the hope that within the broad range of IgG antibody applied to the immunoassay enough specific epitopes will be present to bind enough heterophile molecules in the patient sample to allow for a sufficient sensitivity or specificity for the particular assay. Thus, current heterophile antibody "blocking" approaches do not specifically target the heterophile antibody that may interfere with a particular immunoassay reagent system. Such an approach is needed to allow more sensitivity or specificity in immunoassay test results.

[0013] The problem described above is not confined to immunoassay interference by heterophile molecules. For

example, monoclonal antibodies have been used to enhance delivery of diagnostic and/or therapeutic agents to assess, diagnose, treat and/or prevent disease or a particular condition. By way of example, immunoconjugates, comprising an anticancer agent conjugated to a monoclonal antibody that is specific for the particular type of cancer cells at issue, may be prepared and administered to the patient by known routes and methods. Significantly, immunoconjugates may comprise a number of diagnostic and/or therapeutic agents as readily recognized by those skilled in the art. Typical agents used against cancer cells are radioactive isotopes, drugs, toxins and enzymes.

[0014] Attempts to improve the efficacy of the exemplary anticancer immunoconjugate have been made by administering a protective dose of non-specific blocking antibodies capable of non-specific and/or cross-reactive binding with non-target cells, tissues and/or antigens encountered along the therapeutic pathway, together with the immunoconjugate comprising antibodies specific with the target cells or tissues. See, e.g., U.S. Pat. No. 5,648,059 to Gray, incorporated herein in its entirety by reference. In this context, the non-target interfering cells, tissues and/or antigens may comprise cells, combinations of cells, tissues, amino acids or functional constituent or derivative thereof, polypeptides, interfering antibodies, e.g., heterophile antibodies, and the like. This prior art protective dose is designed to ensure to the extent possible, maximum dosage delivery of the active immunoconjugate agent at the target cell or tissue, by minimizing inactivating binding, interference, and or non-target retention of the immunoconjugate. The protective effect is, however, minimized due to the non-specific nature of the approach.

[0015] Known blocking antibodies used in the prior art protective dose may be administered prior to the active therapeutic and/or diagnostic agent or concurrently. If used as a component of a delivery vehicle for diagnostic or therapeutic immunoconjugates or other agents, known non-specific blocking antibodies may be associated with the active agent by any method known to those skilled in the art. By way of example, the active agent may be combined with the non-specific blocking antibody. One skilled in the art will readily recognize additional technologies for combining a diagnostically and/or therapeutically active agent with such blocking antibodies.

[0016] The non-specific nature of the blocking antibodies used in prior art protective dosing results in an inability to predict efficacy of the protective dose and, as a result, an inability to accurately predict the active agent's dose that is actually delivered to the target. Thus, it is desirable to design and deliver blocking antibodies that specifically mimic the carrier as protection against unknown non-target cells, tissues, antigens and/or antibodies or antibody fragments to enhance therapeutic dosing of immunoconjugate agents or other therapeutic agents susceptible to the effects of non-target binding.

BRIEF SUMMARY OF THE INVENTION

[0017] A specific blocking reagent, method of manufacture thereof, methods of use, and an immunoassay reagent system and method are provided. The blocking reagent uses the specific antibody structure that both recognizes the target, e.g., analyte, and is recognized by any interfering substances, e.g., heterophile molecules. The blocking reagent is, however, a partially inactivated form of the target-specific antibody. Thus, the blocking reagent cannot bind the target because the target-binding regions have been specifically inactivated. The blocking reagent can, however, bind interfering substances such as heterophile molecules as these binding regions remain active. In this manner, the blocking reagent provides highly specific protection from interferents. The blocking reagent thus has broad applicability in immunoassays as well as enhanced delivery of therapeutic and/or diagnostic agents.

[0018] An object of the present invention is to provide a method of manufacturing a specific blocker reagent.

[0019] Another object of the present invention is to provide a blocker reagent that will specifically bind with and block interfering substances such as heterophile molecules contained in a patient sample.

[0020] Another object of the present invention is to provide an immunoassay reagent system comprising a specific blocker reagent designed to specifically bind and block heterophile molecules from interfering with the immunoassay.

[0021] Another object of the present invention is to provide an immunoassay method that utilizes a specific blocker reagent to bind with and block heterophile molecules that may be contained in the patient test sample from interfering with the immunoassay.

[0022] Another object of the present invention is to provide a method for improving delivery of agent(s) to a target in a subject by administering a specific blocker reagent to bind with and block specific interfering substances, e.g., non-target cells, tissues and/or antigens encountered along the therapeutic pathway.

[0023] Another object of the present invention is to provide a pharmaceutical composition to improve delivery of diagnostic and/or therapeutic agents to a target in a subject.

[0024] The figures and the detailed description which follow more particularly exemplify these and other embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The invention may be more completely understood in consideration of the following detailed description of various embodiments of the invention in connection with the accompanying drawings, which are as follows.

[0026] FIG. **1** is an illustration of a typical prior art sandwich immunoassay.

[0027] FIGS. **2**A and **2**B illustrate the effects of heterophile interference in prior art assays.

[0028] FIG. **3** is an illustration of successful prior art heterophile blocking technique.

[0029] FIG. **4** illustrates failure of prior art heterophile blocking technique.

[0030] FIG. **5** is a flowchart for a method of manufacturing one embodiment of the invention.

[0031] FIG. **6** illustrates one embodiment of the exemplary manufacturing method and resulting product.

[0032] FIG. **7** provides illustration of one embodiment of the invention used in an immunoassay.

DETAILED DESCRIPTION OF THE INVENTION

[0033] While the invention is amenable to various modifications and alternative forms, specifics thereof are shown by way of example in the drawings and described in detail herein. It should be understood, however, that the intention is not to limit the invention to the particular embodiments described. On the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention.

[0034] Referring now to FIG. **1**, a typical sandwich-type immunoassay **10** is provided. Thus, a paramagnetic particle **12** is illustrated supporting a target-specific capture antibody **14** and mixed with a patient sample containing the target, e.g., an analyte **20** and a target-specific signal antibody **30**.

[0035] The capture antibody comprises at least one target, e.g., analyte, binding region **15**, and at least one heterophile interferent binding region **16**A, **16**B, **16**C. Each of the binding regions are active in FIG. **1**, meaning that each analyte binding region **15** is capable of binding analyte **20** and each heterophile interferent binding region **16**A, **16**B, **16**C is capable of binding heterophile interferents. The target-specific binding regions **15** are shown having the same structural shape in the Figure, indicating for descriptive and illustrative purposes that the two binding regions **15** shown have an affinity and are capable of binding a specific epitope on one particular target analyte. Similarly, the target-specific signal antibody is illustrated with two target binding regions **31** having the same structural shape, indicating affinity and a capability for binding an epitope on one specific target analyte.

[0036] Moreover, the heterophile binding regions **16**A, **16**B, **16**C on the target-specific capture antibody **14**, are shown having differing structural shapes, indicating an affinity and a capability for being bound by heterophile molecules having structural features corresponding with, and complementary to, the heterophile binding regions **16**A, **16**B, **16**C. Similarly, the target-specific signal antibody **30**, is shown with heterophile binding regions, **33**A, **33**B and **33**C, each having an affinity and a capability for being bound by heterophile molecules having structural features correspondent with binding regions **33**A, **33**B or **33**C, respectively.

[0037] The signal antibody **30** further comprises a signal generating label **32** that is conjugated to the signal antibody **30** by known methods.

[0038] Accordingly, the target or analyte **20** is shown with two binding regions, or epitopes. The first binding region **22** has an affinity for binding within the at least one analyte binding region **15** on the capture antibody **14**. The second binding region or epitope **21** has an affinity. for binding with the at least one corresponding analyte binding region **31** on the signal antibody **30**.

[0039] The paramagnetic particle-supported target-specific antibody **14**, analyte **20**, and target-specific signal antibody **30** are incubated under time and temperature conditions sufficient to allow binding or complexing to occur. In this exemplary embodiment, there are no interfering substances, e.g., heterophile molecules. Thus, complex **50** forms as desired, comprising the paramagnetic particle-supported specific antibody **14** bound with the analyte **20** which is in turn bound with the signal antibody **30**. Structurally, the analyte binding region **15** of the capture antibody **14** is bound with epitope **22** of the analyte while epitope **21** of the analyte is bound with binding region **31** of the signal antibody, forming complex **50**.

[0040] Following incubation, the mixture is washed and all unbound material is eliminated, i.e., any free analyte **20** and/or free signal antibody **30**. The material bound to the paramagnetic particle-supported specific antibody **14**, i.e., complex **50**, is not washed away and is evaluated by any number of known means, e.g., chemiluminescence, to generate a measurable signal and provide either a qualitative or quantitative result for the analyte and with an inherent specificity and sensitivity, terms well known to those skilled in the art.

[0041] Turning to FIGS. **2**A and **2**B, prior art immunoassays, such as those discussed above in connection with FIG. **1**, are subject to interference by a variety of interfering substances, e.g., heterophilic molecules. Heterophiles are capable of interfering with assays in two basic ways: false positive results as illustrated in FIG. **2**A and false negative results as shown in FIG. **2**B.

[0042] FIG. **2**A thus illustrates a false positive interferent complex **60** comprising the paramagnetic particle-supported specific antibody **14** and the signal antibody **30** bridged and bound by a heterophile molecule **35**. In this exemplary embodiment of the false positive complex **60**, the heterophile binding region **16**B of the capture antibody **14** and the heterophile binding region **33**B of the signal antibody **30** have sufficiently similar structural features such that both are bound by the heterophile binding regions **36**A. It is particularly noteworthy that there is no analyte in this particular illustration; thus a negative signal is expected.

[0043] However, as seen in FIG. **2**A, this form of heterophile interference results in an effective bridging of the specific antibody **14** and the signal antibody **30** including signal **32**, in the absence of any analyte. Accordingly, evaluation of this false positive complex **60** generates a positive signal, falsely indicative of the presence of analyte.

[0044] Alternatively, as illustrated in FIG. **2**B a false negative interferent complex **70** may form, comprising the paramagnetic particle-supported specific antibody **14** and a heterophile antibody **35** bound thereto. In this case, one of the heterophile binding regions **36**B is bound to the capture antibody heterophile binding region **16**A, preventing the analyte **20** present in the patient sample from binding to the analyte binding region **15** and providing a falsely negative signal.

[0045] This falsely negative interferent complex **70** is formed not only to the exclusion of the analyte **20** but also the signal antibody **30**. Thus, upon washing, the interferent complex **70** remains and the unbound analyte **20** and unbound signal antibody **30** with label **32** are both eliminated. Evaluation of complex **70** provides a falsely negative signal, and a false negative response.

[0046] Obviously, either form of heterophile interference (false positive or negative) has an adverse effect on the accuracy, specificity or sensitivity of the immunoassay

result. A typical prior art approach to eliminating the interfering effects of heterophile is provided in FIG. **3**. This illustration provides a theoretical example of successful blocking of heterophiles, and as a result, minimization or reduction of the effects of heterophile interference.

[**0047**] In FIG. **3**, the paramagnetic particle-supported specific antibody **14** is mixed with the target-specific signal antibody **30** and the patient sample comprising only heterophile molecules **35** and, in this case, no target analyte. In addition, an exemplary range of non-specific "normal IgG" antibodies **80**A, **80**B, **80**C, **80**D each with different binding regions **81**A, **81**B, **81**C, **81**D, respectively, are provided. Each of the binding regions **81**A, **81**B, **81**C, **81**D are illustrated as having distinct structural shapes indicating binding affinity for specific substances. The "normal IgG" antibodies **80**A, **80**B, **80**C, **80**D are added to the mixture to "flood" the patient sample in an effort to bind and block the problematic heterophiles. The heterophiles **35** in this exemplary case comprise binding regions **36**A with a specific affinity and binding capability, illustrated by structural shape.

[**0048**] This mixture is incubated and complex **90** forms as desired, comprising a heterophile molecule **35** and one particular IgG antibody type **80**A. Note that the particular heterophile **35** illustrated comprises a binding region **36**A with specificity (indicated by complementary structural shaping) for heterophile binding region **16**B on the capture antibody **14** and/or heterophile binding region **33**B on the signal antibody **30**. Thus, without the "normal IgG" blocker approach, the heterophiles **35** may interfere by binding either the capture antibody **14** or the signal antibody **30**, or both. The IgG antibodies **80**A act, at least theoretically, as a molecular sink in that they bind all heterophiles **35** with binding regions **36**A, and thus prevent those particular heterophiles from otherwise interfering with the capture **14** or signal **30** antibodies.

[**0049**] Following washing, the heterophile complex **90** is eliminated along with other unbound material such as unbound IgGs **80**B, **80**C, **80**D and the signal antibodies **30**. In this exemplary case, since there was no target analyte present in the patient sample, the material remaining comprises the paramagnetic particle-supported capture antibody **14**, without analyte or signal antibody **30**. Thus, evaluation of the washed and retained material results in a true negative response.

[**0050**] Of course, in practice, the broad range IgG heterophile "blocking" approach provided in FIG. **3** is quite limited due to the non-specific nature of the IgG antibodies utilized. It is impossible using current techniques, for example, to ensure or even predict whether the correct form of IgG antibody is being used in combination with the specific antibodies employed in the immunoassay.

[**0051**] In contrast, FIG. **4** illustrates the limitations of the prior art IgG heterophile "flooding" blocking approach. Here, as in FIG. **3**, the paramagnetic particle-supported specific antibody **14** is combined with the signal antibody **30** and the patient sample comprising heterophile molecules **35** having a particular binding region **36**B and no analyte. In addition, a broad range of non-specific "normal IgG" antibodies **80**A, **80**B, **80**C, **80**D having binding regions **81**A, **81**B, **81**C, and **81**D, respectively, is added to the mixture and incubated.

[**0052**] Following incubation, complex **100** forms, comprising the specific capture antibody **14** and the signal antibody **30**, bridged by a heterophile molecule **35**, similar to the undesirable falsely positive complex formed in FIG. **2**A. The binding regions **36**B of the heterophile **35** bind with the heterophile binding regions **16**C and **33**A of the capture antibody **14** and the signal antibody **30**, respectively.

[**0053**] Significantly, none of the IgG "blocker" binding regions **80**A, **80**B, **80**C, and **80**D on antibodies **80**A, **80**B, **80**C and **80**D, respectively, have affinity (again illustrated by complementary structural shapes) and binding capability for the heterophile binding regions **36**B present in the exemplary patient sample. As a result, heterophiles **35** not bound by the IgG antibodies **80**A, **80**B, **80**C, **80**D are free to bind and interfere with the capture **14** and/or signal **30** antibodies and the "blocking" attempt fails.

[**0054**] Washing of the mixture results in retention of complex **100** and elimination of all other material. Evaluation of complex **100** yields a falsely positive result since the signal antibody **30**, with signal-generating moiety **32** conjugated thereto, generates a signal in the complete absence of any analyte in complex **100**.

[**0055**] A primary difficulty with the prior art "flooding" IgG blocker approach is that it cannot be predicted whether the result is inaccurately skewed in a falsely positive or negative direction since both forms of interference may occur simultaneously. Indeed, compounding the difficulty is that the nature of the molecules involved indicate that false positive and false negative results may occur simultaneously within the same assay. Thus, it is impossible to create an adjustment to compensate for the existence of such interference. Clearly, a more specific method and reagent for eliminating interfering substances, e.g., heterophile molecules, is needed.

[**0056**] One embodiment of the inventive method of manufacturing a blocker reagent specifically designed to block, i.e., specifically bind and inactivate interfering substances such as heterophile molecules is provided in FIG. **5**.

[**0057**] The method **200** begins with selection of a specific antibody **210**. The antibody selected is an analyte-specific antibody used in the particular immunoassay. In the instance of a sandwich immunoassay, either the analyte-specific capture antibody or the analyte-specific signal antibody, or both, may be selected for use in the method.

[**0058**] Next, the epitopic structure, or binding region, on the target, e.g., analyte targeted by the selected specific antibody, is identified **220**. This may be done by a number of methods known to those skilled in the art. For example, one approach may employ "epitope mapping", in which oligopeptide fragments representing relevant amino acid sequences of the protein target are probed for their ability to interact with the specific antibody.

[**0059**] Once the targeted binding region structure is identified, the structure itself is copied **230**. This is accomplished by any method well known to those skilled in the art. For example, one approach may employ solid phase peptide synthesis followed by HPLC purification, as needed.

[**0060**] The copies are next derivatized to yield reactive intermediate structures **240**, by methods well known to those skilled in the art. In a preferred embodiment, a heterobi-

functional reagent may be used wherein the copies are mixed with the heterobifunctional reagent to yield such reactive derivatized copies.

[0061] Next, the reactive derivatized copies from step **240** are bound to the analyte binding region of the selected specific antibody in step **250**. In a preferred embodiment, the activated copies are mixed with the selected specific antibody from step **210**. The resulting complex comprises the selected specific antibody with reactive copies bound to the analyte binding region(s) of the antibody.

[0062] Finally, the specific antibody selected in **210** is inactivated in step **260**. In the preferred embodiment, the complex from step **250** is subjected to conditions that cause a reaction between the second moiety of the heterobifunctional reagent and the specific antibody, e.g., irradiated with light or other method as known to those skilled in the art. The inactivation reaction renders the target analyte binding regions inactivate, wherein the target analyte is prevented from binding thereto, but the heterophile interferent binding sites remain active.

[0063] Inactivation of the selected specific antibody or antibodies may occur by other methods, each of which is well within the scope of the invention. Generally, inactivation is achieved by at least one modification of the active binding region. For example, one or more specific amino acids in the analyte binding region may be modified by methods known to those skilled in the art, e.g., broad chemistry approaches. Alternatively, at least one substance may be bound with the selected antibody's analyte binding region(s). This substance bound with the analyte binding regions(s) may comprise at least one peptide. The peptide may be derivatized to inactivate the target analyte binding regions. Derivatization of the peptide may not be required, however, to achieve the desired inactivation. Another inactivation approach is to facilitate the binding of at least one derivatizing reagent, e.g., a heterobifunctional reagent or peptide, within the target analyte binding regions to achieve inactivation. The derivatizing reagent may further comprise at least one amino acid residue.

[0064] Turning now to FIG. **6**, one embodiment of the method of manufacture is illustrated, resulting in the inventive blocker reagent.

[0065] Thus, the specific antibody is selected according to the preferred method. In the example, the specific signal antibody **30** in an immunoassay is the antibody chosen. Alternatively, the capture antibody may have been selected. In this particular instance, the antibody **30** has not been conjugated to include the signal element, though the signal element will not interfere with the blocking reagent function and may be present in other embodiments. Further, the selected antibody **30** comprises at least one analyte binding region **31**, specific for the target or analyte's epitopic structure.

[0066] The epitopic structure **21** of the target analyte **20** is identified and copies are made of the identified binding region or epitopic structure **21**. In a specific but not limiting example, the epitopic structure **21** can be an oligopeptide sequence within a polypeptide analyte **20**.

[0067] The copies **21** are mixed with a derivatizing reagent **294** to provide reactive intermediates **296**, capable of binding to the analyte binding region **31** of the selected antibody **30**.

[0068] The reactive intermediates **296** are mixed with the active form of the selected specific antibody **30** to form a pre-blocker complex **298**, wherein one reactive intermediate copy of the targeted epitopic structure **296** is bound to one of the specific antibody analyte binding regions **31**. Preferably, all specific antibody analyte binding regions **31** bind a reactive target structure.

[0069] The pre-blocker complex **298** is then inactivated. Preferably, a second reaction, e.g., irradiation of a photoactivatable functionality with light, or other method as known to those skilled in the art, is used to complete the inactivation to produce the inventive specific blocker **300**. The blocker's analyte-specific binding regions **31** are now inactive and cannot bind analyte **20**; the heterophile interferent binding regions **33**A, **33**B, **33**C remain active and capable of binding heterophile molecules.

[0070] It is understood that the inactivation of the target analyte binding region(s) of the selected specific antibody may result in the complete or partial inactivation of the analyte binding sites. Inactivation is defined in this context as inhibition of the binding of the analyte to the analyte binding region. Thus, "inactive" analyte binding regions cannot bind the targeted analyte.

[0071] Moreover, the inactivation method may change the structure of the selected specific antibody in some manner that does not affect the ability of the inactivated specific antibody to function as intended in binding with the heterophile molecules or other interfering substances. For example, the inactivation process may alter or otherwise affect the analyte binding site(s) and/or the heterophile binding site(s) or other regions of the inactivated antibody. In this connection, "active" heterophile binding site(s) are defined as retaining the ability to bind the heterophile, despite structural or other changes to the inactivated antibody. Thus, the structure of the inventive blocker may be identical, or substantially identical, to the structure of the active specific antibody and be within the scope of the present invention.

[0072] It is further understood that the analyte-specific binding region(s) of the blocker is inactivated while the interfering substance, e.g., heterophile, binding region(s) remain active. For descriptive purposes herein, the blocker is referred to herein as inactivated, even though the interfering substance, e.g., heterophile, binding region(s) remains active.

[0073] FIG. **7** provides one embodiment of the inventive blocker reagent used in an immunoassay reagent system and corresponding method to quantitatively or qualitatively analyze at least one target analyte in a test sample that may, or may not, contain interfering substances, e.g., heterophiles.

[0074] An amount of capture antibody **14**, bound to the paramagnetic particle **12**, is mixed with a patient sample comprising heterophiles **35** without analyte, the inventive blocker reagent **300**, and the signal antibody **30**. The capture antibody **14** comprises heterophile binding regions **16**A, **16**B, **16**C, signal antibody **30** comprises heterophile binding regions **33**A, **33**B, **33**C, and heterophile **35** comprises binding region **36**B in this case. Binding region **36**B is illustrated as having an affinity and ability to bind heterophile binding regions **16**C and/or **33**A. Significantly, heterophile binding region **36**B may have capability to bind one or more binding regions on either the capture antibody **14**, the signal antibody **30**, or both.

[0075] Blocker reagent **300**, an inactivated form of the selected specific antibody, in this case the signal antibody **30**, is shown having three heterophile binding regions **330**A, **330**B and **330**C, corresponding to heterophile binding regions **33**A, **33**B and **33**C of the signal antibody **30**. The signal antibody **30** comprises active analyte binding region(s) **31**. The blocker **300** comprises inactive analyte binding regions(s) **310**A. Thus, heterophiles **35** able to bind the blocker's heterophile binding region **330**A (but not **330**B or **330**C) will bind thereto, while the analyte, when present, cannot bind to the analyte binding region(s) **310**A. It is understood by those skilled in the art that the blocker reagent illustrated and described herein is one of many equivalent forms of molecules, all of which are well within the scope of the present invention.

[0076] The mixture is incubated and complex **400** forms as desired, comprising heterophile **35** bound to, in this exemplary embodiment, two of the inventive blocker molecules **300**. Thus, heterophile binding region **36**B is bound with the structurally corresponding heterophile binding region **330**A on the blocker **300**. Formation of complex **400** effectively and specifically blocks heterophiles **35** from affecting the immunoassay by binding in a specific manner the heterophiles that otherwise would interfere with either the capture antibody **14** and/or the signal antibody **30**.

[0077] Following washing, all material not bound to a paramagnetic particle **12** is eliminated, including complex **400** and the signal antibody **30**. Thus, the paramagnetic particle-supported capture antibody **14** remains for evaluation. Evaluation, in this case, yields a true negative result, either quantitative or qualitative, depending upon the type of evaluation utilized. Because there was no target analyte in the patient sample, the negative result is true and accurate.

[0078] FIG. **7** provides one embodiment wherein the blocker **300** is added simultaneously with the capture antibody **14**, patient sample and signal antibody **30**. Alternatively, the patient sample may be pretreated with the blocker **300** by premixing patient sample with blocker **300** prior to adding the capture antibody **14** and the signal antibody **30**. This allows the blocker **300** to bind and inactivate any potentially interfering heterophiles before addition of the capture **14** and signal **30** antibodies, effectively eliminating competition between the heterophile/blocker, heterophile/capture antibody and heterophile/signal antibody binding possibilities.

[0079] The inventive blocker reagent and immunoassay reagent system and method is effective with all patient samples, including, inter alia, whole blood, plasma, serum, saliva, cerebral spinal fluid, urine, amniotic fluid, interstitial fluid, feces, mucus, cell extracts and tissue extracts. In addition, the blocker reagent and immunoassay method will work in any known immunoassay reagent system, including inter alia, nephelometric, turbidimetric and radiolabeled assay formats.

[0080] The blocker reagent described herein has broad application beyond reduction of interference in immunoassays. For example, monoclonal antibodies are used to enhance delivery of diagnostic and/or therapeutic agents to assess, diagnose, treat and/or prevent disease or a particular condition. By way of example, immunoconjugates, comprising an anticancer agent conjugated to a monoclonal antibody that is specific for a particular type of cancer cell, may be prepared and administered to the patient by known routes and methods. Significantly, immunoconjugates may comprise a number of diagnostic and/or therapeutic agents as readily recognized by those skilled in the art. Typical agents used against cancer cells are radioactive isotopes, drugs, toxins and enzymes.

[0081] The inventive blocker reagent may increase the efficacy of the exemplary anticancer immunoconjugate by administering a protective dose of blocking antibodies capable of highly specific binding with non-target cells, tissues and/or antigens encountered along the therapeutic pathway. The blocking reagent may be administered prior to the active immunoconjugate, simultaneously with, or in combination as a pharmaceutical composition. In this context, the non-target interfering substances, e.g., cells, tissues and/or antigens may comprise cells, combinations of cells, tissues, amino acids or functional constituent(s) or derivative(s) thereof, polypeptides, interfering antibodies, e.g., heterophile antibodies, and the like. The inventive blocker reagent may thus provide a highly specific protective dose that is designed to ensure to the extent possible, maximum dosage delivery of the active immunoconjugate agent at the target cell or tissue, by minimizing inactivating binding, interference, and or non-target retention of the immunoconjugate. The particular active agent or immunoconjugate protected by the blocker reagent is not, of course, limited to anticancer agents. Those skilled in the art will readily recognize the wide range of therapeutic and/or diagnostic agents that will benefit from use of the inventive blocker reagent.

[0082] The inventive blocker reagent when used as a protective dose as described herein may be administered prior to the active therapeutic and/or diagnostic agent or concurrently. If used as a component of a delivery vehicle for active agents, the blocker reagent may be associated with the active agent by any method known to those skilled in the art. By way of example, the active agent may be combined with the blocker reagent. In either case, a pharmaceutical composition comprising the blocker reagent and the active agent may be manufactured and administered to the subject.

[0083] The present invention should not be considered limited to the particular examples described above, but rather should be understood to cover all aspects of the invention. Various modifications, equivalent processes, as well as numerous structures to which the present invention may be applicable will be readily apparent to those of skill in the art to which the present invention is directed upon review of the present specification.

What is claimed is:

1. An immunoassay reagent system to qualitatively or quantitatively analyze at least one analyte in a test sample that might contain heterophile interferents, comprising:

at least one analyte-specific antibody; and

at least one heterophile blocker comprising an inactivated form of the at least one analyte-specific antibody, wherein the analyte is blocked from binding with the heterophile blocker.

2. The immunoassay reagent system of claim 1, wherein the at least one heterophile blocker further comprises at least one inactive analyte binding region, wherein the at least one analyte cannot bind with the blocker.

3. The immunoassay reagent system of claim 1, wherein the at least one heterophile blocker further comprises at least one active heterophile interferent binding region, wherein the at least one heterophile interferent may bind with the blocker.

4. The immunoassay reagent system of claim 1, wherein the at least one heterophile blocker further comprises at least one inactivated analyte binding region and at least one active heterophile interferent binding region.

5. The immunoassay reagent system of claim 2, wherein the at least one heterophile blocker further comprises at least one modification within the at least one analyte binding region to inactivate the at least one analyte binding region.

6. The immunoassay reagent system of claim 2, wherein the at least one heterophile blocker further comprises at least one substance bound with the at least one analyte binding region to inactivate the at least one binding region.

7. The immunoassay reagent system of claim 6, wherein the at least one substance further comprises at least one peptide to inactivate the at least one analyte binding region.

8. The immunoassay reagent system of claim 7, wherein the at least one peptide is derivatized.

9. The immunoassay reagent system of claim 6, wherein the at least one heterophile blocker further comprises at least one derivatizing reagent bound to the at least one binding region to inactivate the at least one binding region.

10. The immunoassay reagent system of claim 9, wherein the at least one derivatizing reagent comprises at least one amino acid residue.

11. A blocker reagent comprising an inactivated form of a target-specific antibody for use in reducing interference of the binding of a target analyte with the target-specific antibody or from the formation of a complex that would be formed in the absence of the target analyte causing a false positive result.

12. The blocker of claim 11, further comprising at least one inactive target analyte binding region, wherein the target analyte is blocked from binding with the blocker.

13. The blocker of claim 11, further comprising at least one active interfering substance binding region, wherein the at least one interfering substance can bind with the blocker.

14. The blocker of claim 12, further comprising at least one active interfering substance binding region, wherein the at least one interfering substance may bind with the blocker.

15. The blocker of claim 12, further comprising at least one modification within the at least one target analyte binding region to inactivate the at least one target analyte binding region.

16. The blocker of claim 12, further comprising at least one substance bound with the at least one target analyte binding region to inactivate the at least one target analyte binding region.

17. The blocker of claim 16, wherein the at least one substance further comprises at least one peptide to inactivate the at least one target analyte binding region.

18. The blocker of claim 12, wherein the target-specific antibody further comprises at least one derivatizing reagent bound with the at least one target analyte binding region to inactivate the at least one target analyte binding region.

19. The blocker of claim 18, wherein the at least one derivatizing reagent comprises at least one amino acid residue.

20. An immunoassay method for quantitatively or qualitatively analyzing at least one analyte in a test sample that may contain heterophilic interferents, comprising:

(a) forming a reaction complex by combining:

(i) a test sample;

(ii) at least one analyte-specific antibody; and

(iii) at least one heterophile blocker, wherein the blocker comprises an inactive form of the at least one analyte-specific antibody and wherein the at least one analyte-specific antibody is blocked from binding to the analyte in the test sample; and

(b) measuring the reaction complex to quantitatively or qualitatively determine the amount or presence of the analyte in the test sample.

21. The immunoassay method of claim 20, further comprising pretreating the test sample prior to forming the reaction complex to remove heterophile interferents by combining the at least one heterophile blocker with the test sample.

22. The immunoassay method of claim 20, wherein the test sample is selected from the group consisting of whole blood, plasma, serum, saliva, cerebral spinal fluid, urine, amniotic fluid, interstitial fluid, feces, mucus, cell extracts and tissue extracts.

23. The immunoassay method of claim 20, further comprising reducing the rate of false positive or false negative test results occurring from heterophilic interference.

24. The immunoassay method of claim 20, further comprising improving the sensitivity or specificity of the immunoassay by reducing heterophilic interference.

25. A method of manufacturing a heterophile interferent blocker reagent for use in an immunoassay reagent system, comprising:

a. selecting at least one antibody specific for an analyte;

b. determining the presence of at least one analyte binding region on the at least one antibody; and

c. inactivating the at least one analyte binding region, thereby blocking the at least one analyte from binding with the at least one analyte binding region.

26. The method of claim 25, wherein the inactivating further comprises modifying the at least one analyte binding region to block the at least one analyte from binding with the analyte binding region.

27. The method of claim 25, wherein the inactivating further comprises binding at least one substance to the at least one analyte binding region.

28. The method of claim 27, wherein the at least one substance comprises at least one peptide.

29. The method of claim 28, wherein the at least one peptide is derivatized.

30. The method of claim 27, wherein the at least one substance comprises at least one derivatizing reagent bound with the at least one analyte binding region.

31. The method of claim 30, wherein the at least one derivatizing reagent comprises at least one amino acid.

32. A method for improving delivery of at least one agent to a target in a subject, wherein at least one interfering substance interferes with delivery of the at least one agent to the target, comprising:

(a) providing at least one immunoconjugate, comprising the at least one agent and a target-specific antibody;

(b) preparing at least one specific blocker reagent that is an inactivated form of the target-specific antibody, wherein the target cannot bind, but the at least one interfering substance can bind;

(c) administering the at least one specific blocker reagent which, when bound with the at least one interfering substance, reduces or eliminates the interference; and

(d) administering the at least one agent concurrently with, or subsequent to, administering the blocker reagent.

33. The method of claim 32, further comprising:

combining the blocker reagent with the immunoconjugate to form a pharmaceutical composition; and

administering the pharmaceutical composition to the subject.

34. A pharmaceutical composition, the composition having a target, comprising:

at least one immunoconjugate comprising at least one target-specific antibody and at least one agent; and

at least one specific blocker, wherein the at least one specific blocker comprises an inactivated form of the at least one target-specific antibody.

* * * * *

专利名称(译)	用于减少嗜异性干扰的阻断剂		
公开(公告)号	US20070184501A1	公开(公告)日	2007-08-09
申请号	US11/349444	申请日	2006-02-07
[标]申请(专利权)人(译)	贝克曼考尔特公司		
申请(专利权)人(译)	BECKMAN COULTER，INC.		
当前申请(专利权)人(译)	BECKMAN COULTER，INC.		
[标]发明人	ODEGAARD BRUCE H		
发明人	ODEGAARD, BRUCE H.		
IPC分类号	G01N33/53		
CPC分类号	G01N33/5306		
外部链接	Espacenet USPTO		

摘要(译)

提供了特定的封闭试剂，其制备方法和使用方法。封闭试剂使用靶识别的特异性抗体结构，例如分析物，和任何干扰物质，例如嗜异性分子。然而，封闭试剂是靶特异性抗体的灭活形式。因此，阻断试剂不能结合靶，因为靶结合区已经失活。然而，阻断试剂可以阻断诸如嗜异性分子的干扰物质，因为这些结合区域保持活性。以这种方式，封闭试剂提供高度特异性的干扰物去除。因此，封闭试剂在免疫测定中具有广泛的适用性以及增强的治疗和/或诊断剂的递送。