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(54) **PHOSPHOROTHIOATE OLIGONUCLEOTIDE-LABELING OF WHITE BLOOD CELLS**

PHOSPHOROTHIOAT- OLIGONUKLEOTID-LABELING VON LEUKOZYTEN

MARQUAGE DES LEUCOCYTES AVEC DES OLIGONUCLÉOTIDES PHOSPHOROTHIOATE

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US-B2- 7 338 762

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Description

BACKGROUND OF THE INVENTION

[0001] The present invention relates to the field of cell analysis. Phosphorothioate oligonucleotides (PS-ODN) are a variant of normal DNA in which one of the non-bridging oxygens is replaced by a sulfur atom.

DESCRIPTION OF RELATED ART

[0002] Labeling of white blood cells (WBC), or leukocytes (also spelled "leucocytes"), using a binding compound that binds to all white blood cells is useful both in the analysis of cells by, for example, flow cytometry and microscopy, and in the enrichment or separation of white blood cells from, for example, whole blood. Typically, an antibody is used that specifically binds to a cell surface protein that is expressed by all WBC, such as CD45. CD45 (also known as leukocyte common antigen, GP180, or T200) is found on all hematopoietic cells except those of red cell origin.

[0003] For cell analysis, anti-CD45 antibody is labeled with a detectable label, e.g., a fluorescent dye, to facilitate detection of the cells. The antibodies are mixed with the sample containing white blood cells, such that the cells become fluorescently labeled through the binding of the antibody to the cell-surface marker. The labeled cells then can be identified and enumerated using an instrument, such as a flow cytometer or a microscope. Dye-labeled anti-CD45 antibodies are commercially available from, for example, BD Biosciences (San Jose, CA). For enrichment or separation, the anti-CD45 antibody typically is conjugated to a solid surface, such as the surface of magnetic particles. The antibody-conjugated magnetic particles are mixed with the sample containing white blood cells, such that the cells become magnetically labeled through the binding of the antibody to the cell-surface marker. The magnetically labeled cells can be concentrated by placing the sample in a magnetic field.

[0004] Typically, the magnetically labeled cells are washed to separate the cells from the sample. Magnetic nanoparticles conjugated to anti-CD45 antibodies are commercially available from, for example, BD Biosciences (San Jose, CA). B. Jahrsdorfer, et al. 2005. *J. Immun. Method.* 297: 259-263 describe a study that demonstrates that phosphorothioate oligodeoxynucleotides (PS-ODN) suppress non-specific binding of Cy5-based monoclonal antibody conjugates, including those containing PE-Cy5 and Cy5 alone, to monocytes and macrophages. This suppression appears to be mediated by PS-ODN binding to CD64, thereby blocking the binding of Cy5 to this receptor. They conclude that PS-ODN can be used as a reagent to prevent nonspecific binding of Cy5-based immune-conjugates to monocytes in flow cytometry assays. E. R. Kandimalla, et al. 2001. *Bioorg. Med. Chem.* 9: 807-813, describe CpG oligonucleotides and their synthesis. CpG oligonucleotides are used as

stimulatory molecules for the activation of certain cells of the immune system.

BRIEF SUMMARY OF THE INVENTION

[0005] The invention is referred to in the claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0006]

Figure 1 shows schematically a method for the modification and labeling of cytosine bases in PS-ODN with FITC.

Figure 2 shows schematically a reaction for the modification and labeling of the 5' end of PSODN with FITC.

Figure 3 shows data demonstrating the binding of PS-ODN#2 to total white blood cells. Leukocyte subpopulations of granulocytes, monocytes, and lymphocytes are labeled as G, M and L, respectively.

Figure 4 shows data demonstrating the binding of PS-ODN#5 to total white blood cells. Leukocyte subpopulations of granulocytes, monocytes, and lymphocytes are labeled as G, M and L, respectively.

Figure 5 shows data from an analysis of cells stained with FITC-PS-ODN#2 and CD19-PE-Cy7.

Figure 6 shows data from an analysis of cells stained with FITC-PS-ODN#5 and CD19-PE-Cy7.

Figure 7 shows data demonstrating the magnetic enrichment of white blood cells from a sample of whole blood using PS-ODN-IMag particles and magnetic separation.

DETAILED DESCRIPTION OF THE INVENTION.

[0007] In order that the invention herein described may be fully understood, a number of terms are explicitly defined, below. Terms not explicitly defined are intended to have their usual meaning in the fields of microscopy, cytometry, immunology, and biology, in general.

[0008] The terms, white blood cells, leukocytes, and leucocytes are used interchangeably herein. By "whole blood" is intended a fluid blood sample as drawn in the presence of an anticoagulant from a mammal and substantially unfractionated thereafter.

[0009] A "labeling reagent", as used herein, refers to any reagent that comprises a detectable moiety and is capable of binding to an analyte of interest, e.g., a cell, to obtain a labeled analyte. Typically, a detection reaction of the present invention will comprise a PS-ODN conjugated to a detectable label, such as a fluorescent dye. The binding of a dye-bound PS-ODN to leukocytes results in the leukocytes becoming labeled with the dye.

[0010] A "capture reagent", as used herein, refers to any reagent that capable of binding to an analyte of interest, e.g., a cell, and that is either bound to, or is capable

of binding to, a solid surface. Typically, a detection reaction of the present invention will comprise a PS-ODN conjugated to a solid surface. The binding of a dye-bound PS-ODN to leukocytes results in the leukocytes becoming immobilized on the solid surface.

[0011] The present invention involves using labeling and capture reagents that comprise phosphorothioate oligonucleotides (PS-ODN). The PS-ODN of this invention may bind to all white blood cells (leukocytes) in an indiscriminative fashion, and enable the labeling, capture, or concentration of leukocytes in a manner that preserves the antigenic integrity of the cells. Particles or dyes may be conjugated directly to the PS-ODN or, preferably, is conjugated to the PS-ODN through a linker. The present invention provides methods of capturing, enriching, or concentrating leukocytes, comprising the steps of contacting a fluid containing leukocytes with PS-ODN bound to a solid surface under conditions such that the leukocytes become bound to the PS-ODN and are thereby captured by the solid surface. Preferably, the surface captured leukocytes are separated from the fluid for further analysis or use. Further analysis or use may comprise contacting the captured leukocytes with monocyte immuno-conjugates. The use of surface-bound capture reagents, in general, to capture, enrich, or concentrate cells is well known in the art. The capture reagents are suitable for use in any of these known methods. The present invention also involves using reagents for the magnetic enrichment or separation of leukocytes. The reagents may comprise a magnetically sensitive particle conjugated to phosphorothioate oligonucleotides (PS-ODN).

[0012] The magnetically sensitive particle or capture reagent may be conjugated directly to the PS-ODN or, preferably, conjugated to the PS-ODN through a linker. The present invention provides methods of magnetically enriching leukocytes, comprising the steps of adding PS-ODN-conjugated magnetic particles to a fluid containing leukocytes under conditions such that the PS-ODN conjugated magnetic particles become bound to leukocytes, and applying a magnetic field to localized magnetically labeled leukocytes.

[0013] Preferably, the magnetically labeled or captured leukocytes are separated from the fluid for further analysis or use. In some embodiments further analysis or use of the concentrated or captured cells may comprise binding the concentrated or separated cells to any known leukocyte immuno-conjugates such as granulocyte, monocyte, or lymphocyte (e.g. anti-CD 19, anti-CD56, anti-CD 19, anti-CD 20, anti-CD 8, anti-CD 4, anti-CD 33, anti-CD 45 etc). The methods of this invention beneficially provide for concentrating or separating leukocytes while preserving antigenic specificity to markers of interest.

Phosphorothioate oligonucleotides (PS-ODN)

[0014] Phosphorothioate oligonucleotides (PS-ODN)

and their synthesis have been described in the literature. For example, see US Patent Nos. 5,644,048 and 5,571,902, and Jahrsdörfer et al., 2005, J. Immun. Method. 297: 259-263. The synthesis methods described in these references are suitable for use in generating the PS-ODN of the present invention.

[0015] PS-ODN molecules of any length may be suitable for use as detection or capture reagents in the present invention. Preferably the length may be between 15 and 30, more preferably between 15 and 25, nucleotides in length. Earlier studies on different PS-ODN sequences (Table 1) suggested that sequence specificity is not a determining factor or pre-requisite for binding to white blood cells, thus any sequence of any length PS-ODN may be incorporated in the methods and reagents. The PS-ODN reagents may have one or more phosphorothioate linkages in combination with one or more conventional phosphate linkages. In a preferred embodiment, the PS-ODN comprises a sequence selected from ODN#1, ODN#2, ODN#3, ODN#4, and ODN#5, shown in Table 1, below. In more preferred embodiments, the PS-ODN is selected from ODN#1, ODN#2, ODN#3, ODN#4, and ODN#5, shown in Table 1, below. More preferably, the PS-ODN is either ODN#2 or ODN#5.

Table 1

[0016] The asterisk (*) indicates phosphorothioate bond between nucleotides.

ODN#1

T*C*G*T*C*G*T*T*T*G*T*C*G*T*T*G*T*C*G*T*
*T

ODN#2

T*G*C*T*G*C*T*T*T*G*T*G*C*T*T*T*G*T*G*C*T*
*T

ODN#3

G*G*G*G*G*A*C*G*A*T*C*G*T*C*G*G*G*G*G*G

ODN#4

G*G*G*G*G*A*G*C*A*T*G*C*T*G*G*G*G*G*G

ODN#5

T*C*G*T*C*G*T*T*T*G*G*C*G*C*G*C*G*C*G

[0017] Not to be bound by theory, the data suggest that PS-ODN bind to carbohydrates and proteins on the cell surface in a non-specific manner, and that the presence of thiol in the phosphodiester backbone of the PS-ODN is involved in the non-specific binding to cell surface. Comparisons of different PS-ODN sequences (Table 1) suggest that sequence specificity is not a determining factor or pre-requisite for binding to white blood cells. As described in the examples, below, PS-ODNs labeled with fluorescein bind to total white blood cells, including lymphocytes, monocytes and granulocytes, indiscriminately. Use of PS-ODN labeled at the 5' end or at cytosine bases provided equivalent results, which indicates that neither the 5' end nor the cytosine bases are not critical for binding of PS-ODN to the cell surface of white blood cells.

Detection Reagents

[0018] The fluorescent detection reagents comprise PS-ODN conjugated to a detectable label, such as a fluorescent dye (fluorophore). Fluorescent dyes (fluorophores) suitable for use in the present invention can be selected from any of the many dyes suitable for use in biological assays, including flow cytometry and imaging applications. However, the invention is not limited to the use of fluorescent dyes, and any of the detection reagents useful in an optical analysis method that bind to the analyte of interest and enable optical detection, such as nanoparticles detectable by surface-enhanced Raman scattering (SERS), may be used.

[0019] Fluorescent dyes are known to those of skill in the art and are commercially available from a number of sources. Suitable fluorescent dyes include, but are not limited to, phycoerythrin ("PE"), fluorescein isothiocyanate ("FITC"), allophycocyanin ("APC"), Texas Red ("TR", Molecular Probes, Inc.), peridinin chlorophyll complex ("PerCp"), CY5 (Biological Detection System) and conjugates thereof coupled to PE (e.g., PE/CY5, PE/APC and PE/TR); etc. A large number of dyes are commercially available from a variety of sources, such as, for example, Molecular Probes (Eugene, OR) and Exciton (Dayton, OH). Detection reagents can be conjugated to PS-ODN using any of a number of well-known methods of conjugating labels to oligonucleotides. Two preferred conjugation reactions are shown in figures 1 and 2.

Capture reagents

[0020] Capture reagents comprise a phosphorothioate oligonucleotide (PSODN) conjugated to a solid surface, or capable of being bound to a solid surface. A wide range of solid surfaces can be used in the present invention, including, for example, beads, plates, slides, columns, microfluidic channels, etc. The solid surface may be conjugated directly to the PS-ODN or, preferably, is conjugated to the PS-ODN through a linker. Methods of conjugating nucleic acids to a solid surface are well known in the art. Alternatively, PS-ODN may be bound to one member of a binding pair, and the solid surface bound to the other member of the binding pair, such that the binding of the PS-ODN to the solid surface can be carried out after the binding of the PS-ODN to the cell. Examples of binding pairs that are suitable for binding the PS-ODN to the solid surface are well known in the art and include, for example, antibody and its target antigen pairs, and streptavidin and biotin.

Magnet capture reagent

[0021] The magnetic capture reagents are magnetic particles bound to PSODN. As used herein, "magnetic particles" refers to any particle that contains a magnetic or magnetically responsive material. Magnetic particles can be of any shape, but typically are approximately

spherical ("microspheres"). Magnetic particles suitable for use in the present invention preferably have a diameter in the nanometer to micrometer range, typically about 0.01 to 50 μm in diameter, preferably about 0.1 to 10 μm , more preferably about 0.2 to 0.4 μm . Preferably, the magnetic particles are paramagnetic or superparamagnetic. Magnetic particles suitable for use in the present invention are commercially available from a number of sources, including, but not limited to, BD Biosciences (San Jose, CA), Invitrogen (Carlsbad, CA), Miltenyi Biotec (Bergisch Gladbach, Germany), and Polysciences (Warrington, PA). In general, magnetic particles sold for use in cell separation assays are suitable for use in the present invention. Conjugation of the magnetic particles to PS-ODN can be carried using any of a number of well-known methods of conjugating oligonucleotides to magnetic particles. Magnetic particles are commercially available with reactive surface groups suitable for use in conjugating to oligonucleotides. Alternatively, as with other solid surfaces, the PS-ODN may be bound to one member of a binding pair, and the magnetic particle bound to the other member of the binding pair, such that the binding of the PS-ODN to the magnetic particle can be carried out after the binding of the PS-ODN to the cell.

EXAMPLES

[0022] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed.

Example 1

PS-ODN#2 binding to total white blood cells.

[0023] PS-ODN#2 labeled with FITC, conjugated to cytosine residues, were synthesized as shown in figure 1. 50- μl blood samples were incubated with either 5 μg unlabeled PS-ODN#5 or FITC-PS-ODN#2 for 30 minutes, followed by treatment with BD FACS™ Lysing Solution. Cells were centrifuged, washed, and analyzed on a flow cytometer. The data are shown in figure 3.

[0024] The results show that PS-ODN#2 labeled with FITC bind to all three populations of white blood cells (lymphocytes, monocytes and granulocytes), i.e., all white blood cells. The results further show that PS-ODN can be labeled with FITC at 5' end or at cytosine bases (figure 1 and 2). These modifications (FITC-labeling) either at 5' end or at cytosine bases do not abolish the ability of PS-ODN to bind to cell surface (shown in figure 3 and 4) where the labeled PS-ODNs bind to all three populations of white blood cells.

Example 2

PS-ODN#5 binding to total white blood cells.

[0025] PS-ODN#5 labeled with FITC at the 5'-end were synthesized as shown in figure 2. 50- μ l blood samples were incubated with either 5 μ g unlabeled PS-ODN#5 or FITC-PS-ODN#5 for 30 minutes, followed by treatment with BD FACS™ Lysing Solution. Cells were centrifuged, washed, and analyzed on a flow cytometer. The data are shown in figure 4. The results show that PS-ODN#5 labeled with FITC bind to all three populations of white blood cells (lymphocytes, monocytes and granulocytes), i.e., all white blood cells. The results further show that PS-ODN can be labeled with FITC at 5' end or at cytosine bases (figure 1 and 2). These modifications (FITC-labeling) either at 5'end or at cytosine bases do not abolish the ability of PS-ODN to bind to cell surface (shown in figure 3 and 4) where the labeled PS-ODNs bind to all three populations of white blood cells.

Example 3

Staining cells with FITC-PS-ODN#2 and CD19-PE-Cy7.

[0026] A 50- μ l blood sample was incubated with 5 μ g FITC-PS-ODN#2 and 150 ng anti-CD19-PECy7 for 30 minutes, followed by treatment with BD FACS™ Lysing Solution. Cells were centrifuged, washed, and analyzed on a flow cytometer. The data are shown in figure 5. The data indicate that the labeled PS-ODN binds to all white blood cells. Furthermore, the binding of the labeled PS-ODN does not mask the specific antigen on the cell surface, as shown by the staining of the cells using labeled CD 19-specific antibody. Thus, binding of PS-ODNs to the cell surface provides a method of staining cells that maintains the antigenic integrity of the cells.

Example 4

Staining cells with FITC-PS-ODN#5 and CD19-PE-Cy7.

[0027] A 50- μ l blood sample was incubated with 5 μ g FITC-PS-ODN#5 and 150 ng anti-CD19-PE5 Cy7 for 30 minutes, followed by treatment with BD FACS™ Lysing Solution. Cells were centrifuged, washed, and analyzed on a flow cytometer. The data are shown in figure 6. The data indicate that the labeled PS-ODN binds to all white blood cells. Furthermore, the binding of the labeled PS-ODN does not mask the specific antigen on the cell surface, as shown by the staining of the cells using labeled CD 19-specific antibody.

Example 5

Magnetic separation of total white blood cells with PS-ODN-IMag.

[0028] Magnetic particles (BD IMag magnetic particles, BD Biosciences, San Jose, CA) were conjugated to PS-ODN#5. A 50- μ l sample of whole blood was incubated with 32 μ g Fe of PS-ODN#5-conjugated magnetic particles for 30 minutes, followed by treatment with BD FACS™ Lysing Solution. Cells were magnetically separated using a BD IMagnet™ cell separation magnet (BD Biosciences, San Jose, CA), washed, and analyzed on a flow cytometer. As a control, a sample of untreated cells was analyzed by flow cytometer. The data are presented in figure 7.

[0029] The results show that PS-ODN-conjugated magnetic particles may be used to enrich or separate white blood cells by any separation method, (e.g., magnetic separation). An advantage of the present methods is that the cell surface markers are left intact for antibody labeling and analysis.

25 **Claims**

1. A method for indiscriminately labeling leukocytes in a whole blood fluid sample, comprising the step of adding a conjugated phosphorothioate oligonucleotide to said sample under conditions such that the conjugated phosphorothioate oligonucleotide becomes bound to the leukocytes.
2. The method of claim 1, further comprising adding an immuno-conjugate to the sample under conditions such that the immune-conjugate becomes bound to the leukocytes.
3. The method of claim 1 or 2, wherein the conjugated phosphorothioate oligonucleotide is conjugated to a fluorescent dye, or wherein the conjugated phosphorothioate oligonucleotide is conjugated to a capture reagent, preferably wherein the capture reagent is magnetic.
4. The method of claim 3, further comprising placing the sample in a magnetic field such that leukocytes bound to the magnetic capture reagents are concentrated.
5. The method of claim 4, further comprising adding an immuno-conjugate to the concentrated leukocytes under conditions such that the immune-conjugate becomes bound to the leukocytes, preferably wherein the immuno-conjugate is an anti-CD19 antibody.
6. The method according to any of the preceding claims wherein the phosphorothioate oligonucleotide is be-

tween 15 and 25 nucleotides in length.

7. The method according to any of the preceding claims wherein the phosphorothioate oligonucleotide (PS-ODN) comprises a sequence selected from the PS-ODN consisting of ODN#1, ODN#2, ODN#3, ODN#4, and ODN#5, shown in Table 1.
8. A whole blood sample containing indiscriminately labelled leukocytes obtainable according to any of the claims 1-7.

Patentansprüche

1. Verfahren zur willkürlichen Markierung von Leukozyten in einer Vollblutflüssigkeitsprobe, umfassend den Schritt der Zugabe eines konjugierten Phosphorothioat-Oligonukleotids zur Probe unter solchen Bedingungen, dass das konjugierte Phosphorothioat-Oligonukleotid an die Leukozyten gebunden wird.
2. Verfahren nach Anspruch 1, ferner umfassend Zugabe eines Immunkonjugats zur Probe unter solchen Bedingungen, dass das Immunkonjugat an die Leukozyten gebunden wird.
3. Verfahren nach Anspruch 1 oder 2, wobei das konjugierte Phosphorothioat-Oligonukleotid an einen Fluoreszenzfarbstoff konjugiert ist oder wobei das konjugierte Phosphorothioat-Oligonukleotid an ein Fängerreagens konjugiert ist, vorzugsweise wobei das Fängerreagens magnetisch ist.
4. Verfahren nach Anspruch 3, ferner umfassend Platzen der Probe in einem magnetischen Feld, so dass an die magnetischen Fängerreagentien gebundene Leukozyten konzentriert werden.
5. Verfahren nach Anspruch 4, ferner umfassend Zugabe eines Immunkonjugats zu den konzentrierten Leukozyten unter solchen Bedingungen, dass das Immunkonjugat an die Leukozyten gebunden wird, vorzugsweise wobei es sich bei dem Immunkonjugat um einen Anti-CD19-Antikörper handelt.
6. Verfahren gemäß einem der vorhergehenden Ansprüche, wobei das Phosphorothioat-Oligonukleotid eine Länge zwischen 15 und 25 Nukleotiden aufweist.
7. Verfahren gemäß einem der vorhergehenden Ansprüche, wobei das Phosphorothioat-Oligonukleotid (PS-ODN) eine Sequenz umfasst, die aus dem PS-ODN bestehend aus ODN#1, ODN#2, ODN#3, ODN#4 und ODN#5, dargestellt in Tabelle 1, ausgewählt ist.

8. Vollblutprobe, enthaltend gemäß einem der Ansprüche 1-7 erhältlich willkürlich markierte Leukozyten.

5 Revendications

1. Procédé de marquage indiscriminé de leucocytes dans un échantillon de fluide de sang total, comprenant l'étape d'ajout d'un conjugué de phosphorothioate oligonucléotide audit échantillon dans des conditions telles que le conjugué de phosphorothioate oligonucléotide devienne lié aux leucocytes.
2. Procédé de la revendication 1, comprenant en outre l'ajout d'un immunoconjugué à l'échantillon dans des conditions telles que l'immunoconjugué devienne lié aux leucocytes.
3. Procédé de la revendication 1 ou 2, dans lequel le conjugué de phosphorothioate oligonucléotide est conjugué à un colorant fluorescent, ou dans lequel le conjugué de phosphorothioate oligonucléotide est conjugué à un réactif de capture, de préférence dans lequel le réactif de capture est magnétique.
4. Procédé de la revendication 3, comprenant en outre le placement de l'échantillon dans un champ magnétique de sorte que les leucocytes liés aux réactifs de capture magnétiques soient concentrés.
5. Procédé de la revendication 4, comprenant en outre l'ajout d'un immunoconjugué aux leucocytes concentrés dans des conditions telles que l'immunoconjugué devienne lié aux leucocytes, de préférence dans lequel l'immunoconjugué est un anticorps anti-CD19.
6. Procédé selon l'une quelconque des revendications précédentes dans lequel le phosphorothioate oligonucléotide a une longueur d'entre 15 et 25 nucléotides.
7. Procédé selon l'une quelconque des revendications précédentes dans lequel le phosphorothioate oligonucléotide (PS-ODN) comprend une séquence choisie parmi les PS-ODN constitués de ODN n° 1, ODN n° 2, ODN n° 3, ODN n° 4 et ODN n° 5, décrits dans le tableau 1.
8. Échantillon de sang total contenant des leucocytes marqués de façon indiscriminée pouvant être obtenus selon l'une quelconque des revendications 1 à 7.

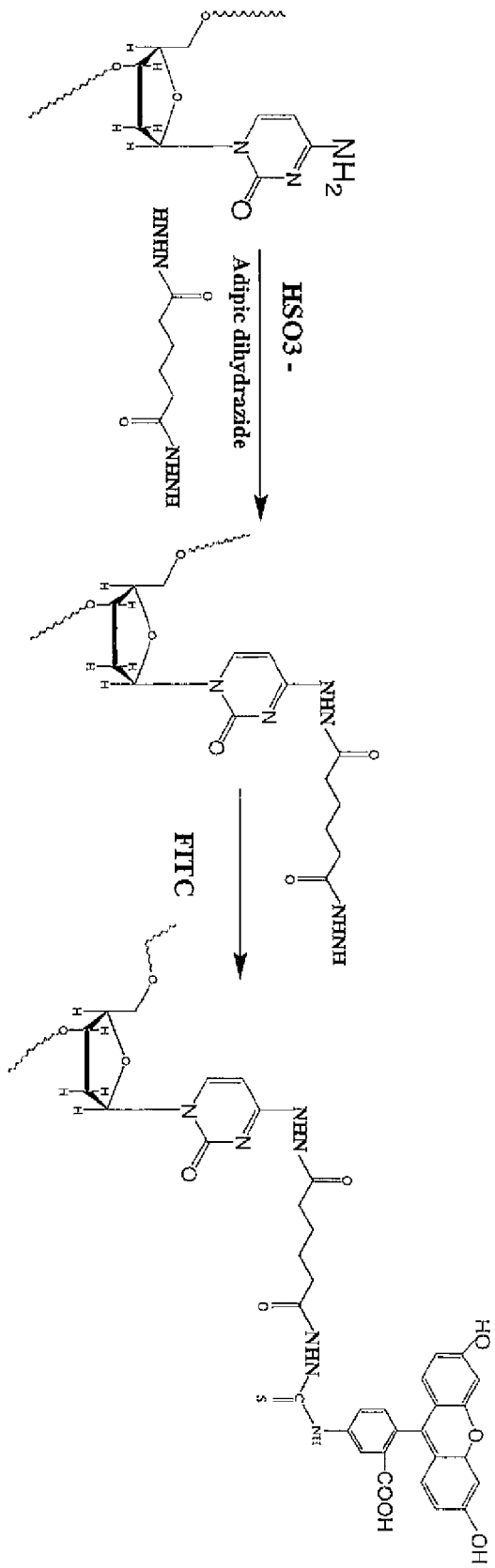


Figure 1
Labeling cytosine bases in PS-ODN with FITC.

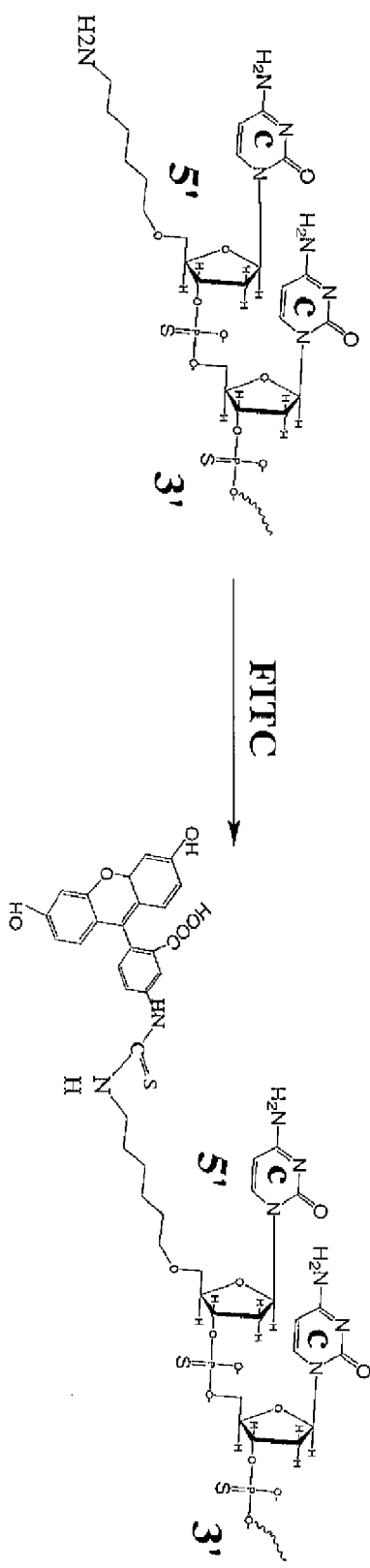


Figure 2
Labeling the 5' end of PS-ODN with FITC.

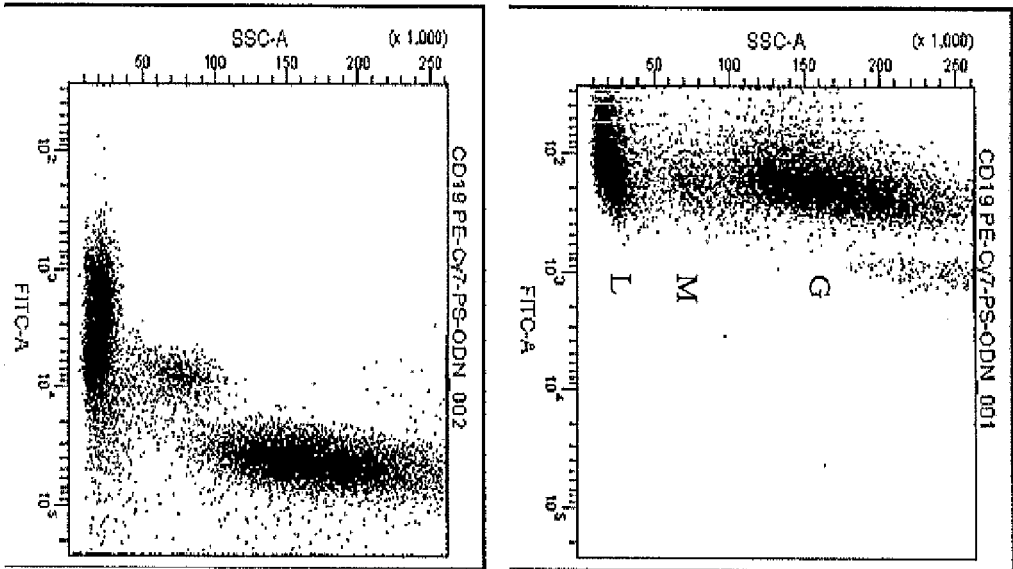


Figure 3

PS-ODN#2 (unlabeled)

PS-ODN#2 labeled with FITC at cytosine bases

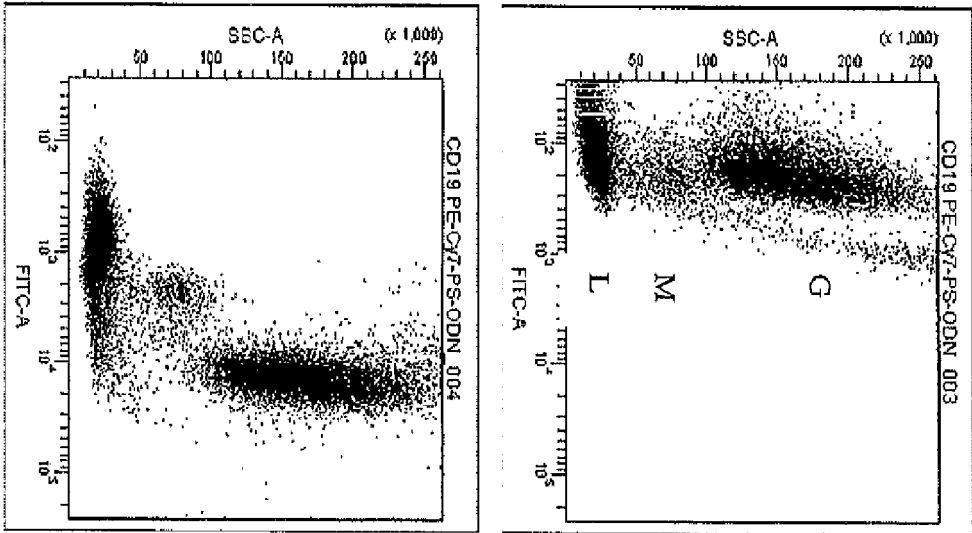


Figure 4

PS-ODN#5 (unlabeled)

PS-ODN#5 labeled with FITC at the 5' end

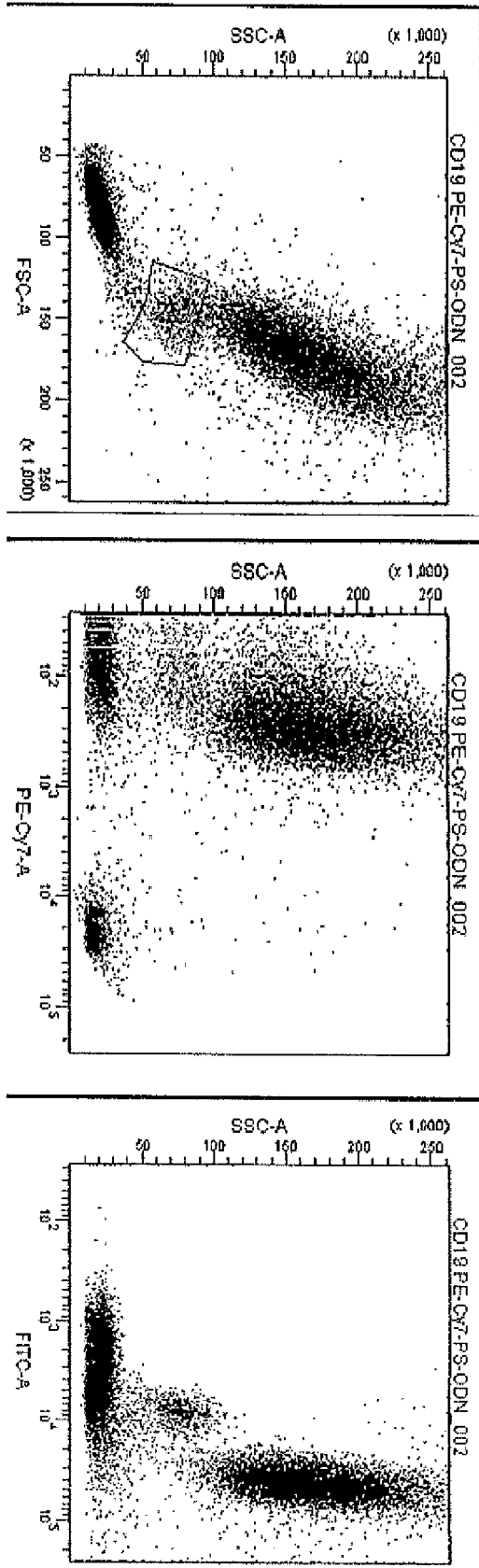


Figure 5
Staining cells with FITC-PS-ODN#2 and CD19-PE-CY7

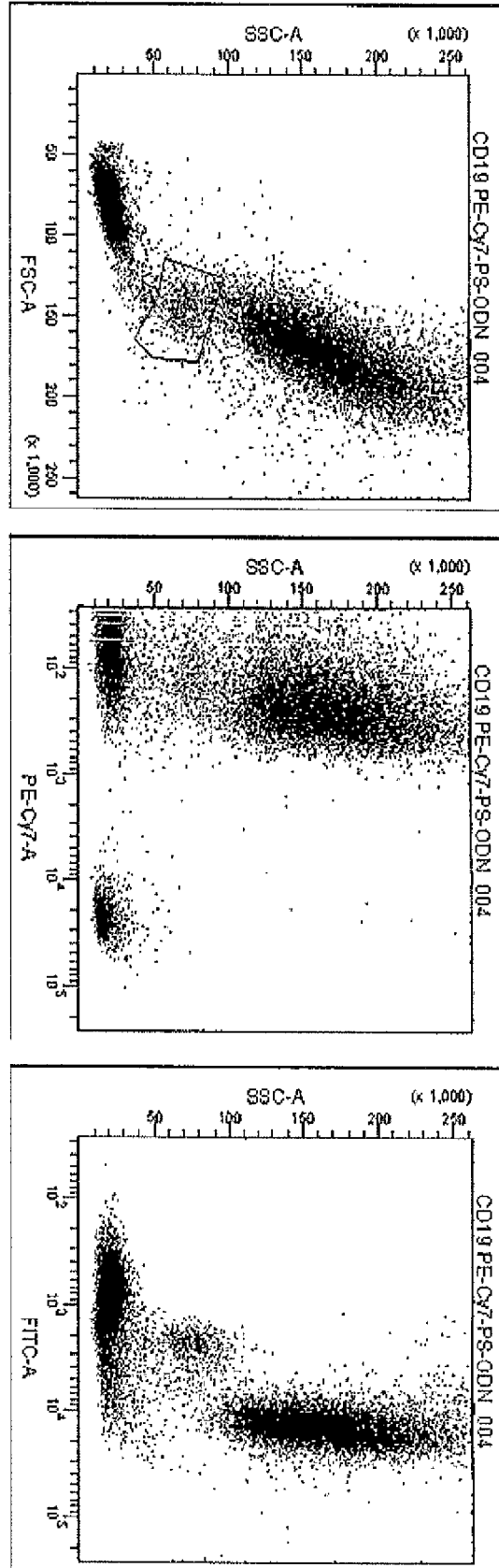


Figure 6
Staining cells with FITC-PS-ODN#5 and CD19-PE-Cy7

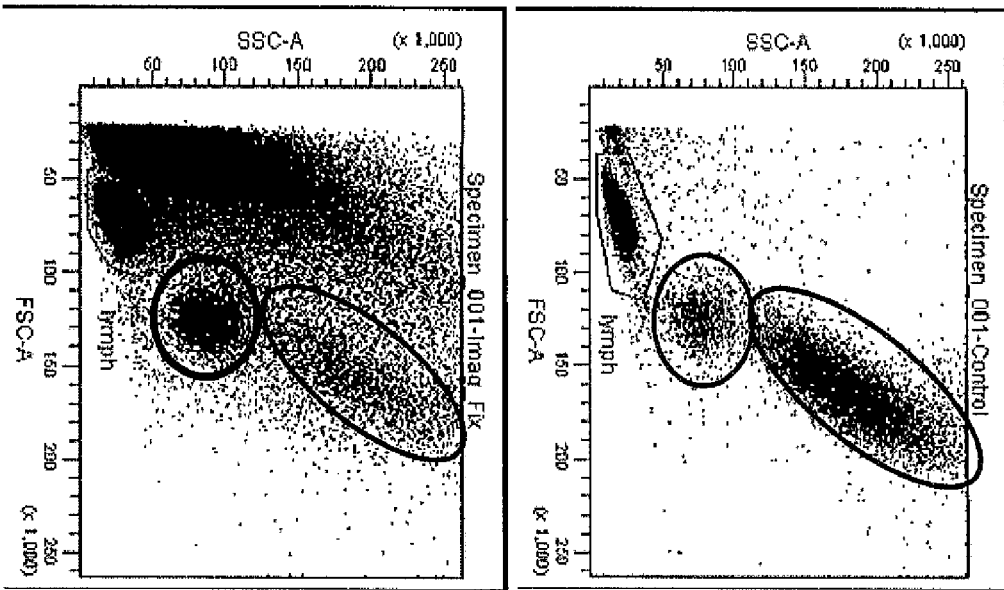


Figure 7

Untreated cells (Control)

Cells treated with PS-ODN-IMag and magnetically separated

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 5644048 A [0014]
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Non-patent literature cited in the description

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- **E. R. KANDIMALLA et al.** *Bioorg. Med. Chem.*, 2001, vol. 9, 807-813 [0004]
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专利名称(译)	硫代磷酸寡核苷酸标记白细胞		
公开(公告)号	EP2795326B1	公开(公告)日	2017-07-05
申请号	EP2012860171	申请日	2012-12-17
[标]申请(专利权)人(译)	贝克顿·迪金森公司		
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IPC分类号	G01N33/531 G01N33/563 C12Q1/68 C12N15/11		
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优先权	61/577502 2011-12-19 US		
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外部链接	Espacenet		

摘要(译)

本发明提供了包含硫代磷酸酯寡核苷酸 (PS-ODN) 的标记和捕获试剂。 PS-ODN以无区别的方式与所有白细胞 (白细胞) 结合, 并以保留细胞抗原完整性的方式标记, 捕获或浓缩白细胞。 提供了使用硫代磷酸酯寡核苷酸的方法。

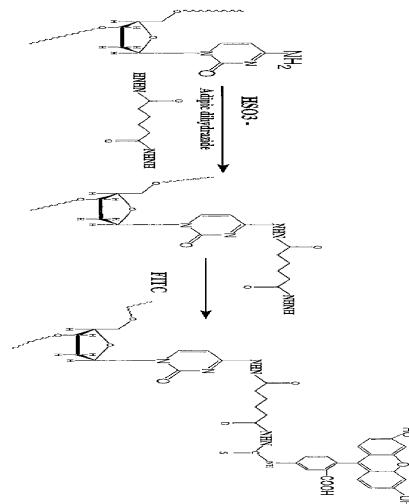


Figure 1
Labeling cytosine bases in PS-ODN with TTC.