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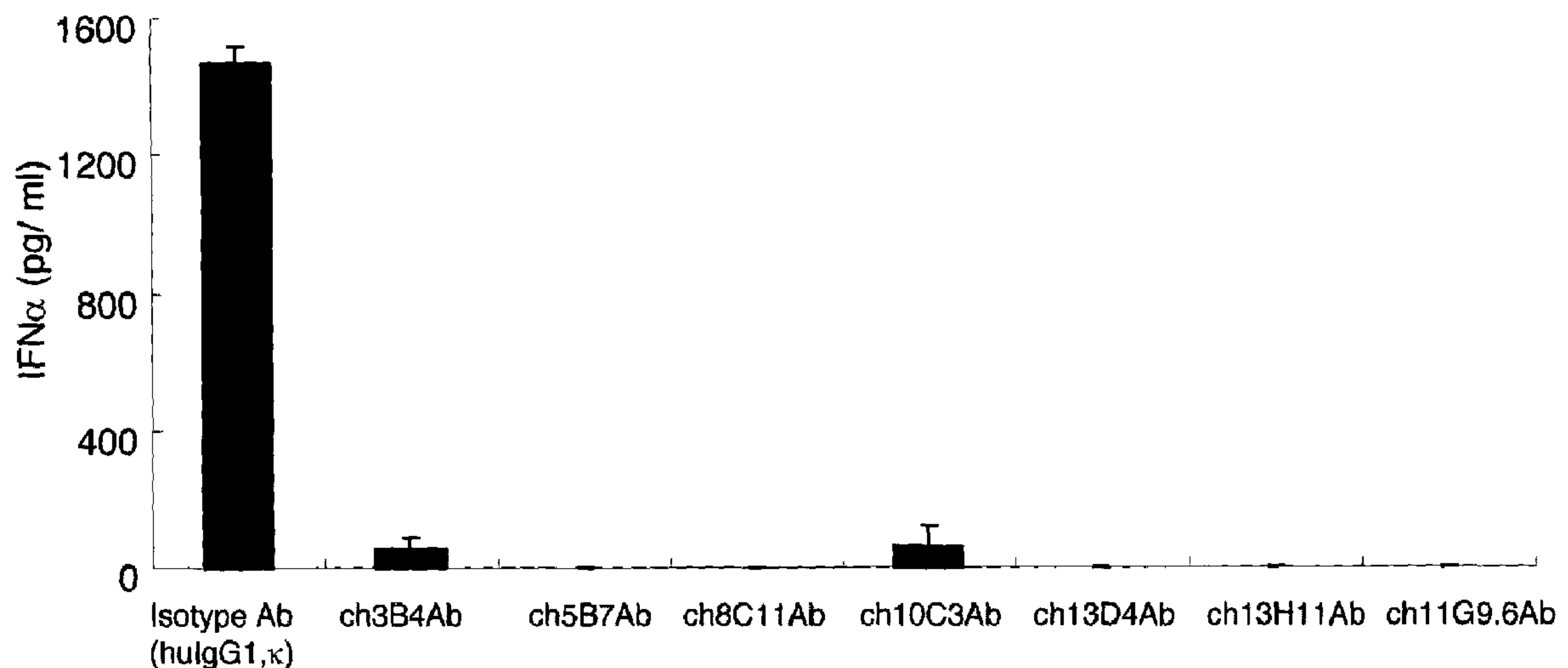
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(54) **Titre : ANTICORPS DIRIGE CONTRE LA PHOSPHOLIPASE D4**

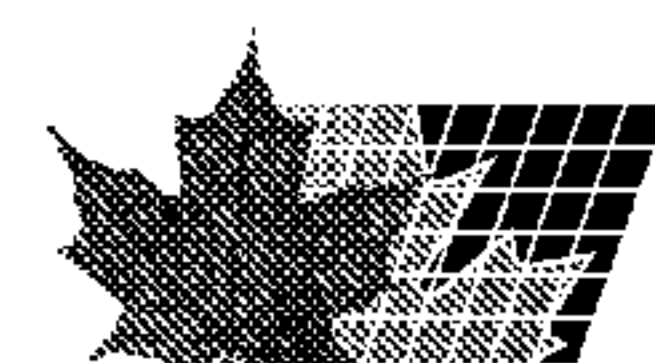
(54) **Title: ANTI-PHOSPHOLIPASE D4 ANTIBODY**

FIG. 26



(57) **Abrégé/Abstract:**

A monoclonal antibody that binds to a phospholipase D4 (PLD4) protein, or a fragment containing an antigen-binding region thereof.



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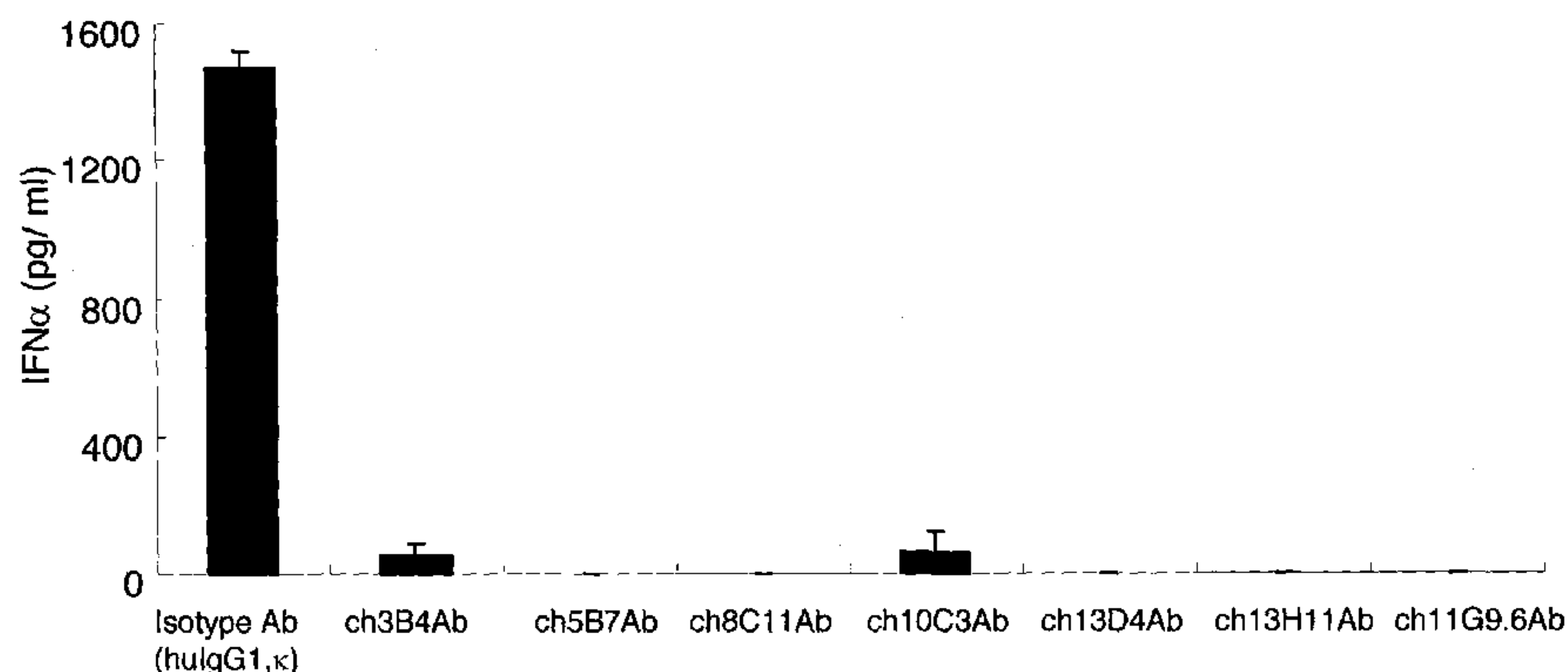
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FIG. 26



(57) Abstract: A monoclonal antibody that binds to a phospholipase D4 (PLD4) protein, or a fragment containing an antigen-binding region thereof.

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DESCRIPTION

ANTI-PHOSPHOLIPASE D4 ANTIBODY

Technical Field

[0001]

The present invention relates to an antibody that binds to phospholipase D4.

Hereinafter, "phospholipase D" may be abbreviated as PLD, and "phospholipase D4" and the like

5 may be abbreviated as PLD4 and the like.

Background Art

[0002]

Interferon (hereinafter, the "interferon" may be abbreviated as IFN) is the most important cytokine in the anti-virus immune response. Interferon producing cell in human

10 blood (IPC: IPC is an undifferentiated lymphocyte-based dendritic cell positioned as a precursor cell of the dendritic cell (DC). The IPC may be also called plasmacytoid dendritic cell or plasmacytoid dendritic cell (pDC). Hereinafter, in the present specification, IPC and pDC are synonymous, and uniformly referred to as a term of pDC in principle below.) expresses CD4 and major histocompatible complex class II protein. However, isolation or particular

15 characterization of the cells has not been performed until now due to an insufficient number of such cells, rapid apoptosis, and further lack of lineage (system) marker. It has been revealed that pDC is CD4⁺CD11c⁻ type precursor cell of the dendritic cell, and found out that pDC produces IFN more by 200 to 1000 folds than other blood cells after stimulation by a microorganism. Accordingly, pDC is a conclusive immune system effector cell in an anti-

20 virus/anti-tumor immune response.

IFN α and IFN β are known as type I IFN having anti-virus activity or anti-tumor activity. On the other hand, it has been revealed that IFN α is associated with autoimmune diseases. For example, abnormal production of IFN α has been reported in patients of autoimmune diseases such as systemic lupus erythematosus and chronic rheumatoid arthritis.

25 Furthermore, it has been reported a case where autoimmune disease symptoms are expressed or aggravated upon administration of a recombinant IFN α 2 or IFN. It has been also suggested that autoimmune symptoms are likely to be alleviated by neutralization of IFN α .

[0003]

In addition, it has been also revealed that IFN α induces differentiation of

dendritic cell (DC). It has been contemplated that induction of differentiation of a dendritic cell constitutes an important mechanism in an autoimmune disease since a dendritic cell is an antigen presenting cell. In fact, it has been suggested that induction of differentiation of a dendritic cell of IFN α is deeply associated with development of systemic lupus erythematosus. As described above, close relationship of IFN α with autoimmune diseases as well as anti-tumor activity has been pointed out. In addition, IFN α is also deeply associated with development of psoriasis.

[0004]

Only a few pDC exists in the blood. It is contemplated that the ratio of pDC occupying the peripheral blood lymphocyte is 1% or less. However, pDC has very high IFN-production ability. The IFN-production ability of pDC reaches, for example, 3000 pg/mL/ 10^4 cells. That is to say, it can be said that most of IFN α or IFN β in the blood produced at the time of virus infection is caused by pDC, although the number of the cells is small.

[0005]

pDC is differentiated into a dendritic cell by virus stimulation, and induces production of IFN- γ or interleukin (IL)-10 by T cell. In addition, pDC is also differentiated into a dendritic cell by IL-3 stimulation. The dendritic cell differentiated upon IL-3 stimulation induces production of Th2 cytokine (IL-4, IL-5, IL-10) by T cell. As described above, pDC has a property that it is differentiated into different dendritic cells depending on the difference of stimulations.

[0006]

Accordingly, pDC is a cell that has two sides, i.e., one side as an IFN producing cell, and the other side as a precursor cell of a dendritic cell. Either one of the cells plays an important role in the immune system. That is to say, pDC is one of the important cells that support the immune system in various aspects.

[0007]

In regulation of the activity of a humoral factor such as IFN, administration of an antibody that recognizes the factor is effective. For example, an attempt to treat autoimmune diseases with an antibody against IL-1 or IL-4 was in practical use. In addition, also for IFN similarly, neutralization antibody is regarded as a therapeutic agent for autoimmune diseases. It can be expected that similar approach is effective for IFN producing pDC. However, such approach is based on inhibition of the action of a humoral factor after being produced. If production of an intended humoral factor can be directly controlled, further essential therapeutic effects can be achieved.

[0008]

Antibodies that recognize human pDC have been reported. For example, anti-BDCA-2 monoclonal antibody is a human pDC-specific monoclonal antibody (Dzionic, A. et al. J. Immunol, 165: 6037-6046, 2000). It has been revealed that the anti-BDCA-2 monoclonal antibody has an action of suppressing IFN production of human pDC (J. Exp. Med. 194: 1823-1834, 2001). Additionally, it has been also reported that a monoclonal antibody that recognizes mouse interferon-producing cell suppresses the production of interferon (Blood 2004 Jun 1; 103/11: 4201-4206. Epub 2003 Dec). It has been also reported that the number of dendritic cells decreases by a monoclonal antibody for mouse pDC (J. Immunol. 2003, 171: 6466-6477). [0009]

Similarly, it would be useful if an antibody that can recognize human pDC and regulate the activity thereof is provided. For example, the present inventors revealed already that an antibody recognizing Ly49Q specifically binds to mouse pDC. However, the antibody for Ly49Q did not interfere with the activity of mouse pDC (Blood, 1 April 2005, Vol. 105, No.7, pp. 2787-2792).

[0010]

PLD is an enzyme that catalyzes a reaction of hydrolysis of phosphatidyl choline to produce phosphatidic acid and choline, and causes signaling in various cells. It is contemplated that the produced phosphatidic acid functions as a lipid signal molecule.

PLD1 and PLD2 are conventionally known as two kinds of mammal PLDs, and contain Phox homology domain (PX domain), which is bondable to phosphatidyl inositide, and pleckstrin homology domain (PH domain) at the N terminal region thereof. Both of the domains are involved in PLD membrane targeting.

PLD1 and PLD2 further contain two His-x-Lys-x-x-x-x-Asp sequences (HKD motif). This HKD motif is an essential domain in PLD activity.

It has been contemplated that phosphatidic acid produced by PLD1 and PLD2 is involved in re-constitution of cellular skeleton, exocytosis, phagocytosis, canceration, cell adhesion, chemotaxis, and the like, and acts centrally in the nerve system, the immune system, and the like.

[0011]

Although human Hu-K4 and mouse SAM9 are officially named as PLD3 until now, they are lack of PX and PH domains, and exhibit no PLD activity though they have two HKD motifs. Furthermore, although there are three PLD family members, i.e., PLD4, PLD5, and PLD6, these nonclassical PLDs are scarcely known.

[0012]

The cerebellar development transcriptome database (CDT-DB) for gene expression pattern in development of mouse cerebellum was searched, and as a result thereof, PLD4, which was a transcription product that was controlled at the time of development, was identified (see Tao et al., Nat. Methods 2(8), 591-598(2005)). Basic characteristics of PLD4 have not been reported. It is regarded that it should be determined from now whether PLD4 exhibits enzymatic activity or not, and whether a de-glycosylated form of PLD4 has PLD activity or not.

[0013]

PLD4 is a 506 amino acid sequence represented by SEQ ID NO: 1 (Tao et al., Nat. Methods 2(8), 591-598(2005) and Clark et al., Genome Res. 13(10), 2265-2270(2003)).

The PLD4 protein has two tentative PDE regions (phosphodiesterase motif), which are constituted with two HKD motifs (amino acid sequence of His-x-Lys-x-x-x-x-Asp, wherein x is the other amino acids) conserved in the C terminal region, and a presumptive phosphorylation site (Thr 472). The structure of the PLD4 protein is predicted as a type II monotropic transmembrane protein. In addition, the N terminal region of the PLD4 protein does not have PX region and PH region, which are possessed by PLD1 and PLD2 that are classical PLD family (FIGFIGS. 1 and 2).

On the other hand, although PLD4 belongs to the PLD family from the fact that it has two HKD motifs, PLD4 is lack of PX domain and PH domain, but has a putative transmembrane domain instead.

[0014]

mRNA expression of PLD4, which was characteristically at from a low level to a medium level, was found in a cell subpopulation that was preferentially localized at the corpus callosum and the periphery of the white matter region including cerebellar white matter of a mouse 1 week after birth. These cells expressing the PLD4 mRNA have been identified as Ibal positive microglia (see Tao et al., Nat. Methods 2(8), 591-598(2005)).

The period of 1 week after birth is a period when activation of myelin formation starts in the corpus callosum and the cerebellar white matter of a mouse. In this period, PLD4 is highly expressed at the amoeboid (activated state) microglia that exists in the white matter. From these facts, a possibility is contemplated that PLD4 expression cell in the white matter is involved in myelin formation in this period. Particularly, accumulation PLD4 in the phagocytic vesicle becomes evident, and a possibility is suggested that PLD4 expression cell is involved in phagocytosis. From the amoeboid microglia in the activated state, various cytokines or growth factors are secreted, and phagocytosis is activated as well. It is contemplated that extra

oligodendrocyte (glia cell in the central nervous system, which forms myelin as rolled and attached to the axon) causes apoptosis in the white matter of the brain at the development stage. A possibility has been contemplated that the extra oligodendrocyte is degraded and removed from the amoeboid microglia to secrete a signal molecule, whereby to arrange the environment for myelin formation in the white matter. It is suggested that PLD4 is involved in these processes including the myelin formation.

[0015]

PLD4 mRNA expression is universally seen also in non-nerve tissues, but is mainly distributed in the spleen. Strong PLD4 expression is detected at the periphery of the border zone of red pulp of the spleen, and splenic PLD4 protein collected from the membrane fraction in the cell is highly N-glycosylated. When PLD4 was expressed in a heterogeneous cell system, they were localized in the endoplasmic reticulum and the Golgi body. Heterologously-expressed PLD4 showed no PLD enzymatic activity (Plos ONE www.plosone.org, November 2010, Volume 5, Issue 11, e13932).

From the pattern of the PLD4 expression limited in terms of time and location, it is suggested that PLD4 plays a role in common functions in the microglia or the cell in the spleen border region at the time of brain development at the initial stage after birth.

[0016]

The PLD4 mRNA expression and PLD4 distribution in the nerve tissue and non-nerve tissue have been overviewed above. However, the present inventors found out that PLD4 mRNA is specifically highly expressed in a pDC cell at the resting stage (resting pDC) in the level of a cell species described below.

Mouse anti-human PLD4 polyclonal antibody against total length human PLD4 protein is commercially available (PLD4 purified MaxPab mouse polyclonal antibody (B01P), catalog No. H00122618-B01P, manufactured by Abnova Corporation). However, a monoclonal antibody that binds only to a certain site of PLD4, or a monoclonal antibody that can specifically bind to PLD4, has not been obtained.

Citation List

Non-Patent Literature

[0017]

(1) Tao et al., Nat. Methods 2(8), 591-598 (2005)

(2) Clark et al., Genome Res. 13(10), 2265-2270 (2003)

(3) Plos ONE www.plosone.org, November 2010, Volume 5, Issue 11, e13932

(4) Catalog of mouse PLD4 polyclonal antibody against full length human PLD4 protein

(Abnova, catalog No. H00122618-B01P)

(5) Dzionek, A. et al. J.Immunol. 165: 6037-6046, 2000

(6) J. Exp. Med.194:1823-1834, 2001

(7) Blood 2004 Jun 1;103/11:4201-4206. Epub 2003 Dec

5 (8) J. Immunol. 2003, 171:6466-6477

(9) Blood, 1 April 2005, Vol. 105, No. 7, pp. 2787-2792

(10) Nat. Methods 2(8), 591-598(2005)

Summary of Invention

Technical Problem

10 [0018]

A problem to be solved by the invention is to provide an antibody that binds to PLD4, and to detect, identify, or isolate pDC. In addition, a problem to be solved by the invention is to regulate activity of pDC.

Solution to Problem

15 [0019]

The present inventors confirmed through a research for PLD4 that expression of PLD4 specifically rises in pDC, particularly pDC at the resting stage in addition to pDC at the active stage. Consequently, the present inventors tried the preparation of PLD4 antibody and the elucidation of its action.

20 [0020]

In order to obtain an antibody that recognizes a slight amount of a protein derived from a living organism, a protein prepared by gene recombinant technology is generally used as an immunogen. The present inventors tried expression of PLD4 based on the information of the base sequence of PLD4 cDNA, and an amino acid sequence (GenBank Accession No.

25 NM_138790.2) encoded by it, which has been already revealed (Nat. Methods 2(8), 591-598 (2005)).

[0021]

In order to obtain an antibody of a protein, use of a partial amino acid sequence of a natural protein as an immunogen is often tried. However, in order for an antibody to
30 recognize a molecule on the cell surface, it is necessary to select a region that constitutes a part recognized by the antibody as an epitope on the cell surface. Accordingly, it has been contemplated that it is not realistic to use a fragment amino acid sequence as an immunogen to

obtain an antibody specific to PLD4.

[0022]

Under such circumstances, the present inventors revealed that use of a special immunogen allows obtaining an antibody that binds to pDC. Furthermore, the present inventors confirmed that thus-obtained antibody specifically recognizes human pDC, and further has an action of regulating its activity, and completed the invention. That is to say, the invention relates to an anti-PLD4 antibody, a preparation method thereof, and use thereof described below.

The invention is as follows:

10 (1) A monoclonal antibody that binds to a phospholipase D4 (PLD4) protein, or a fragment containing an antigen-binding region thereof.

(2) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence SYWMH (SEQ ID NO: 2) as CDR1, the sequence DIYPGSDSTNYNEKFKS (SEQ ID NO: 3) as CDR2, and the
15 sequence GGWLDAMDY (SEQ ID NO: 4) as CDR3 in the heavy chain variable region.

(3) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence RASQDISNYLN (SEQ ID NO: 5) as CDR1, the sequence YTSRLHS (SEQ ID NO: 6) as CDR2, and sequence QQGNTLPW (SEQ ID NO: 7) as CDR3 in the light chain variable region.

20 (4) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence SYWMH as CDR1, the sequence DIYPGSDSTNYNEKFKS as CDR2 and the sequence GGWLDAMDY as CDR3 in the heavy chain variable region, and has the sequence RASQDISNYLN as CDR1, the sequence YTSRLHS as CDR2, and the sequence QQGNTLPW as CDR3 in the light chain variable region.

25 (5) The monoclonal antibody (3B4 antibody), or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence TYWMH (SEQ ID NO: 8) as CDR1, the sequence AIYPGNSETSYNQKFKG (SEQ ID NO: 9) as CDR2, and the sequence GYSDFDY (SEQ ID NO: 10) as CDR3 in the heavy chain variable region.

30 (6) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence HASQGIRSNIG (SEQ ID NO: 11) as CDR1, the sequence HGTNLED (SEQ ID NO: 12) as CDR2, and the sequence VQYVQFP (SEQ ID NO: 13) as CDR3 in the light chain variable region.

(7) The monoclonal antibody, or a fragment containing an antigen-binding region

thereof as described in the above-mentioned (1), which has the sequence TYWMH as CDR1, the sequence AIYPGNSETSYNQKFKG as CDR2, and the sequence GYSDFDY as CDR3 in the heavy chain variable region, and has the sequence HASQGIRSNIG as CDR1, the sequence HGTNLED as CDR2, and the sequence VQYVQFP as CDR3 in the light chain variable region.

5 (8) The monoclonal antibody (5B7 antibody), or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence DYNLH (SEQ ID NO: 14) as CDR1, the sequence YIYPYNGNTGYNQKFKR (SEQ ID NO: 15) as CDR2, and the sequence GGIYDDYYDYAIDY (SEQ ID NO: 16) as CDR3 in the heavy chain variable region.

10 (9) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence RASENIYSHIA (SEQ ID NO: 17) as CDR1, the sequence GATNLAH (SEQ ID NO: 18) as CDR2, and the sequence QHFWGTP (SEQ ID NO: 19) as CDR3 in the light chain variable region.

(10) The monoclonal antibody, or a fragment containing an antigen-binding
15 region thereof as described in the above-mentioned (1), which has the sequence DYNLH as CDR1, the sequence YIYPYNGNTGYNQKFKR as CDR2, and the sequence GGIYDDYYDYAIDY as CDR3 in the heavy chain variable region, and has the sequence RASENIYSHIA as CDR1, the sequence GATNLAH as CDR2, and the sequence QHFWGTP as CDR3 in the light chain variable region.

20 (11) The monoclonal antibody (8C11 antibody), or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence SYLY (SEQ ID NO: 20) as CDR1, the sequence LINPTNSDTIFNEKFKS (SEQ ID NO: 21) as CDR2, and the sequence EGGYGYGPFAY (SEQ ID NO: 22) as CDR3 in the heavy chain variable region.

25 (12) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence TSSQTLVHSNGNTYLH (SEQ ID NO: 23) as CDR1, the sequence KVS NRFS (SEQ ID NO: 24) as CDR2, and the sequence HSTHVP (SEQ ID NO: 25) as CDR3 in the light chain variable region.

30 (13) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence SYLY as CDR1, the sequence LINPTNSDTIFNEKFKS as CDR2, and the sequence EGGYGYGPFAY as CDR3 in the heavy chain variable region, and has the sequence TSSQTLVHSNGNTYLH as CDR1, the sequence KVS NRFS as CDR2, and the sequence HSTHVP as CDR3 in the light

chain variable region.

(14) The monoclonal antibody (10C3 antibody), or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence SYGMS (SEQ ID NO: 26) as CDR1, the sequence TISSGGSYIYYPESVKG (SEQ ID NO: 27) as CDR2, and the sequence LYGGRRGYGLDY (SEQ ID NO: 28) as CDR3 in the heavy chain variable region.

(15) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence RSSKSLHSDGITYLY (SEQ ID NO: 29) as CDR1, the sequence QMSNLAS (SEQ ID NO: 30) as CDR2, and the sequence AQNLEL (SEQ ID NO: 31) as CDR3 in the light chain variable region.

(16) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence SYGMS as CDR1, the sequence TISSGGSYIYYPESVKG as CDR2, and the sequence LYGGRRGYGLDY as CDR3 in the heavy chain variable region, and has the sequence RSSKSLHSDGITYLY as CDR1, the sequence QMSNLAS as CDR2, and the sequence AQNLEL as CDR3 in the light chain variable region.

(17) The monoclonal antibody (13D4 antibody), or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence SHYYWT (SEQ ID NO: 32) as CDR1, the sequence YISYDGSNNYNPSLKN (SEQ ID NO: 33) as CDR2, and the sequence EGPLYYGPNPYWYFDV (SEQ ID NO: 34) as CDR3 in the heavy chain variable region.

(18) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence RASQDIDNYLN (SEQ ID NO: 35) as CDR1, the sequence YTSRLHS (SEQ ID NO: 36) as CDR2, and the sequence QQFNTLP (SEQ ID NO: 37) as CDR3 in the light chain variable region.

(19) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence SHYYWT as CDR1, the sequence YISYDGSNNYNPSLKN as CDR2, and the sequence EGPLYYGPNPYWYFDV as CDR3 in the heavy chain variable region, and has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2, and the sequence QQFNTLP as CDR3 in the light chain variable region.

(20) The monoclonal antibody (13H11), or a fragment containing an antigen-

binding region thereof as described in the above-mentioned (1), which has the sequence SHYYWS (SEQ ID NO: 38) as CDR1, the sequence YISYDGSNNYNPSLKN (SEQ ID NO: 39) as CDR2, and the sequence EGPLYYGNPYWYFDV (SEQ ID NO: 40) as CDR3 in the heavy chain variable region.

5 (21) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence RASQDIDNYLN (SEQ ID NO: 41) as CDR1, the sequence YTSRLHS (SEQ ID NO: 42) as CDR2, and the sequence QQFNTLP (SEQ ID NO: 43) as CDR3 in the light chain variable region.

10 (22) The monoclonal antibody, or a fragment containing an antigen-binding region thereof as described in the above-mentioned (1), which has the sequence SHYYWS as CDR1, the sequence YISYDGSNNYNPSLKN as CDR2, and the sequence EGPLYYGNPYWYFDV as CDR3 in the heavy chain variable region, and has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2, and the sequence QQFNTLP as
15 CDR3 in the light chain variable region.

(23) A monoclonal antibody, or a fragment containing an antigen-binding region thereof, which is produced by any one of hybridomas mp5B7, mp7B4, mp13D4, and mp13H11 that are deposited under Accession Numbers: NITE ABP-1211, NITE ABP-1212, NITE ABP-1213, and NITE ABP-1214.

20 (24) A hybridoma that produces any one of the monoclonal antibodies as described in the above-mentioned (1) or (2).

(25) A hybridoma mp5B7, mp7B4, mp13D4 or mp13H11 that is deposited under Accession Numbers: NITE ABP-1211, NITE ABP-1212, NITE ABP-1213 or NITE ABP-1214.

25 (26) A method of preparing a monoclonal antibody, containing a process of culturing the hybridoma as described in the above-mentioned (25), and collecting a monoclonal antibody from the culture.

(27) A method of preparing a cell that produces a monoclonal antibody that binds to PLD4, containing the following processes;

1) a process of administering recombinant PLD4-Ig fusion protein that encodes an amino acid sequence containing a PLD4 extracellular domain, to an immune animal, and
30

2) a process of selecting an antibody-producing cell that produces an antibody that binds to PLD4 from antibody-producing cells of the immune animal.

(28) The method as described in the above-mentioned (27), wherein the cell that expresses PLD4 is a cell that retains an extrinsic polynucleotide that encodes an amino acid

sequence containing the PLD4 extracellular domain in an expressible way.

(29) The method as described in the above-mentioned (28), wherein the cell is an animal cell.

(30) The method as described in the above-mentioned (29), wherein the cell is a
5 human-derived cell.

(31) The method as described in the above-mentioned (30), wherein the human-derived cell is a HEK-293T cell.

(32) The method as described in any one of the above-mentioned (27) to (31), containing a process of cloning the obtained antibody-producing cells incrementally.

10 (33) A method of preparing a monoclonal antibody that binds to a PLD4 extracellular domain, containing a process of culturing the antibody-producing cells obtained by the method as described in the above-mentioned (29), and collecting a monoclonal antibody from the culture.

(34) A monoclonal antibody that recognizes PLD4, or a fragment containing an
15 antigen-binding region thereof, which can be obtained by the following processes;

1) a process of administering a recombinant PLD4-Ig fusion protein that encodes an amino acid sequence containing a PLD4 extracellular domain, to an immune animal,

2) a process of selecting an antibody-producing cell that produces an antibody that binds to PLD4 from antibody-producing cells of the immune animal, and

20 3) a process of culturing the antibody-producing cells selected in the process 2), and collecting an antibody that recognizes PLD4 from the culture.

(35) An immunogen for preparing an antibody that binds to PLD4, containing (a) an animal cell that retains a polynucleotide that encodes an amino acid sequence containing a PLD4 extracellular domain extrinsically in an expressible way, or a cell membrane fraction
25 thereof.

(36) The immunogen as described in the above-mentioned (35), wherein the animal cell is a human-derived cell.

(37) A method of detecting a plasmacytoid dendritic cell, containing a process of bringing a monoclonal antibody that binds to a PLD4 extracellular domain, or a fragment
30 containing an antigen-binding region thereof into contact with a test cell, and detecting a monoclonal antibody, or a fragment containing an antigen-binding region thereof that binds to the cell.

(38) A reagent for detection of a plasmacytoid dendritic cell, containing a monoclonal antibody that binds to a PLD4 extracellular domain, or a fragment containing an

antigen-binding region thereof.

(39) A method of suppressing an activity of a plasmacytoid dendritic cell, containing a process of bring any one of the components described below into contact with the plasmacytoid dendritic cell:

5 (a) a monoclonal antibody that binds to PLD4 and suppresses an activity of the plasmacytoid dendritic cell, or a fragment containing an antigen-binding region thereof, and

(b) an immunoglobulin in which a complementarity-determining region of the monoclonal antibody (a) is transplanted, or a fragment containing an antigen-binding region thereof.

10 (40) A method of suppressing an activity of a plasmacytoid dendritic cell in a living organism, containing a process of administering any one of the components described below to the living organism:

(a) a monoclonal antibody that binds to PLD4, and suppresses an activity of the plasmacytoid dendritic cell, or a fragment containing an antigen-binding region thereof, and

15 (b) an immunoglobulin in which a complementarity-determining region of the monoclonal antibody (a) is transplanted, or a fragment containing an antigen-binding region thereof.

(41) The method as described in the above-mentioned (39) or (40), wherein the activity of a plasmacytoid dendritic cell is either one of interferon producing activity and survival of an interferon producing cell, or both of them.

(42) An agent for suppressing an activity of a plasmacytoid dendritic cell, containing any one of the components described below as an active component:

(a) a monoclonal antibody that binds to PLD4, and suppresses an activity of the plasmacytoid dendritic cell, or a fragment containing an antigen-binding region thereof, and

25 (b) an immunoglobulin in which a complementarity-determining region of the monoclonal antibody (a) is transplanted, or a fragment containing an antigen-binding region thereof.

(43) The agent for suppressing an activity of an interferon producing cell as described in the above-mentioned (42), wherein the activity of a plasmacytoid dendritic cell is either one of interferon producing activity and survival of an interferon producing cell, or both of them.

Effects of invention

[0023]

The invention provides an antibody that specifically recognizes PLD4, immunity that is useful in preparation of the antibody, and a method of preparing an anti-PLD4 antibody using the immunogen. PLD4 is a membrane protein that belongs to PLD4 family. The present inventors revealed that an antibody that specifically recognizes PLD4 can be easily
5 obtained. The anti-PLD4 antibody that can be obtained by the invention is an antibody having high specificity, which distinguishes human pDC from a cell that expresses other PLD families.
[0024]

In a preferred aspect, the anti-PLD4 antibody provided by the invention binds to human pDC. In addition, the antibody of the invention specifically recognizes human pDC.
10 Accordingly, the antibody of the invention is useful for detection or isolation of pDC. pDC is a cell that produces most of type 1 IFN. Accordingly, such detection or isolation is important in diagnosis or research of diseases associated with pDC such as autoimmune diseases.
[0025]

Furthermore, in a preferred aspect, the anti-PLD4 antibody provided by the
15 invention has an action of regulating human pDC activity. Accordingly, the anti-PLD4 antibody of the invention can be used for suppressing pDC activity. Accordingly, if suppression of pDC activity is employed using the antibody of the invention, therapeutic effects can be expected for a patient of an autoimmune disease in which IFN α expression has risen.
[0026]

20 pDC produces a large amount of IFN in a small number of cells. In neutralization of IFN, an antibody is necessary, which depends on the number of IFN molecules. However, in the invention, the activity of the production cell is directly suppressed. As a result thereof, potent IFN suppression effect can be expected with less amount of the antibody in comparison with neutralization of an anti-IFN antibody. Furthermore, in a case where IFN is
25 produced continuously, it is expected that neutralization by IFN antibody will remain as transient suppression. However, in the invention, pDC activity is suppressed, and from this, IFN-producing suppression effect can be expected over a long time.

Brief Description of Drawings

[0027]

30 FIG. 1 is a diagram that illustrates the amino acid sequence of human PLD4 (C14orf175) protein (506 residues). It is contemplated that the 31 to 53 residues from the N terminal are a transmembrane domain as analyzed using "SOSUI program (http://bp.nuap.nagoya-u.ac.jp/sosui/sosui_submit.html)", which is prediction system for a

transmembrane region. The 54 to 506 residues contain two phosphodiesterase motifs, and this protein is predicted to be type II transmembrane protein;

FIG. 2 is a schematic diagram that represents a predicted structure of the human PLD4 protein. The structure has two HKD (HxKxxxxD) motifs in the amino acid 506 residues,
5 and the residue of threonine 472 is possibly a phosphorylation site;

FIG. 3 is a graph that illustrates the homology of human PLD4 protein with homologous molecular species in a heterogeneous animal. The PLD4 protein has been conserved in the evolution process from a mouse to a human;

FIG. 4 is a graph that illustrates the homology with human PLD4 protein family
10 (paralogous);

FIG. 5 is a graph that illustrates human pDC-specific expression of PLD4 gene in a cell responsible for human immunity. Expression of PLD4 gene is high in pDC at the resting stage, and the expression is low in CD19⁺ B cell;

FIG. 6 is a graph that illustrates the tissue expression pattern of human PLD4
15 mRNA. The expression is high in the spleen and the peripheral blood leucocyte;

FIG. 7 is a schematic diagram that illustrates the structure of recombinant human PLD4-Ig fusion protein. A cDNA fragment that corresponds to human PLD4 extracellular domain (56-506 amino acids) was amplified by PCR. This fragment was inserted into the BamHI-EcoRI cloning site of N-Flag pcDNA3.1 expression vector containing mouse Igk leader
20 segment and mouse IgG2a heavy chain constant Fc region (hinge + CH2 + CH3) at the N terminal. 293F cell was transfected temporarily with a plasmid, and the culture supernatant was collected;

FIG. 8 is a FACS analysis diagram that illustrates binding specificity to human PLD4. The mp11G9.6 and ch11G9.6 antibodies specifically recognized human PLD4, but did
25 not recognize human PLD3-293T or PLD5-293T transfectants;

FIG. 9 is a FACS analysis diagram that illustrates cross-reactivity with cynomolgus monkey PLD4. The mp11G9.6 and ch11G9.6 antibodies were capable of recognizing cynomolgus monkey PLD4 on the 293T transfectant;

FIG. 10 is a FACS measurement diagram that illustrates staining in the mp11G9.6
30 antibody of human PBMC. The mp11G9.6 antibody strongly recognized BDCA2⁺ pDC in human PBMC;

FIG. 11 is a FACS analysis diagram that illustrates staining in the purified anti-PLD4 antibody of CAL-1 cell;

FIG. 12 is a FACS analysis diagram that illustrates staining in anti-PLD4 antibody

of human PLD4-CT125 stable cell strain;

FIG. 13 is a FACS analysis diagram that illustrates staining in anti-PLD4 antibody of human PBMC. All of the anti-PLD4 antibodies were capable of recognizing BDCA2⁺ pDC in human PBMC;

5 FIG. 14 is a diagram that represents the multiple alignment, and the homology of the proteins of human PLD, cynomolgus monkey PLD4, rhesus monkey PLD4, and mouse PLD4;

FIG. 15 is a FACS analysis diagram that illustrates staining in anti-PLD4 antibody with transient gene introduction of Flag tagged cynomolgus monkey PLD4 expression vector
10 into human PLD4-293T cell. Cell surface expression of the cynomolgus monkey PLD4 protein was confirmed in the anti-Flag antibody;

FIG. 16 is a FACS measurement diagram that illustrates staining in anti-PLD4 antibody of cynomolgus monkey PLD4-CT125 cell. Among the anti-PLD4 antibodies, seven (3B4, 5B7, 7B4, 13D4, 13H11, 14C1, and 11G9.6) antibodies can binds to cynomolgus monkey
15 PLD4-CT125 stable transfectant;

FIG. 17 is a FACS analysis diagram that illustrates staining in anti-PLD4 antibody, with transient gene introduction of Flag tagged rhesus monkey PLD4 expression vector into human PLD4-293T cell. Cell surface expression of the rhesus monkey PLD4 protein was confirmed in the anti-Flag antibody;

20 FIG. 18-1 and FIG. 18-2 are FACS analysis diagrams that illustrate staining in anti-PLD4 antibody of rhesus monkey PBMC. Among the anti-PLD4 antibodies, five (5B7, 7B4, 13D4, 13H11, and 14C1) antibodies bound specifically to pDC cell population (Lineage-CD123 + HLA-DR+) of cynomolgus monkey in rhesus monkey PBMC;

FIG. 19 is a graph that illustrates dissociation constant molar concentration (nM
25 unit, Kd value) of anti-PLD4 antibody against human PLD4-CT125 stable cell strain;

FIG. 20 is a graph that illustrates CDC activity of ten kinds of the anti-PLD4 antibodies. Target cell: human PLD4-CT125 (mouse 2B4 T cell lymphocyte) stable transfectant antibody concentration: 10 µg/mL effector: 1% immature rabbit complement;

FIG. 21 is a graph that illustrates CDC activity of the anti-PLD4 antibodies
30 (mp11G9.6 antibody and ch11G9.6 antibody). Target cell: human PLD4-CT125 (mouse 2B4 T cell lymphocyte) stable transfectant antibody concentration: 0.1 µg/mL to 30 µg/mL effector: 1% immature rabbit complement;

FIG. 22 is a graph that illustrates ADCC activity of anti-PLD4 chimeric antibody. Target cell: human PLD4-CHO stable transfectant antibody concentration: 10 µg/mL;

FIG. 23 is a graph that illustrates measurement with ELISA of IFN- α secretion inhibition by treatment with anti-PLD4 chimeric antibody (ch11G9.6) in human PBMC isolated from three healthy individuals; and

FIG. 24 is a diagram that illustrates human pDC loss after treatment with anti-PLD4 chimeric antibody.

FIG. 25 is a result of ADCC assay with chimeric anti-PLD4 Abs against human primary pDCs.

FIG. 26 is IFN α production from PBMCs by CpG2216 in the presence of chimeric anti-PLD4 Abs.

10 Mode for Carrying out the Invention

[0028]

The present inventors found that PLD4 is a molecule that is specifically expressed in the mRNA level and protein level in a plasmacytoid dendritic cell at the resting stage (resting pDC). A method of preparing an antibody that recognizes PLD4 is not established.

15 [0029]

There is a report that mouse PLD4 is a molecule that is expressed in amoeboid (activated state) microglia at development stage in the cerebellum or the corpus callosum at the initial stage after birth. However, expression of human PLD4 is not known until now. Particularly, expression in the immune system, intracellular location, structure, function, and the like of human PLD4 have not been reported until now. It was confirmed by the invention that human PLD4, which was contemplated until now to be expressed only in the cytoplasm, is a cell surface marker that is expressed in human plasmacytoid dendritic cell (pDC) as type II transmembrane protein. Accordingly, binding of PLD4 antibody to pDC becomes possible, and it has been proved that PLD4 antibody is useful as a molecular target of a therapeutic antibody intended to regulate functions of B cell and pDC cell.

25 [0030]

The present inventors confirmed by gene expression analysis that PLD4 is specifically expressed in human pDC. It was contemplated that if an antibody that can distinguish PLD4 immunologically from other molecules, is obtained, it would be useful for pDC research. However, there are many molecules in the PLD family including PLD4, which are very similar in the structure to each other. Molecules such as PLD1, PLD2, PLD3, and PLD5, including PLD that is PLD4, encompass an amino acid sequence having particularly high homology (FIG. 4). Accordingly, it was contemplated that it is difficult to obtain an antibody

that distinguish these molecules mutually using an immunogen of a peptide that employs an amino acid sequence (a partial sequence) that constitutes PLD4 (or extracellular domain).

Consequently, the present inventors tried acquisition of an antibody against PLD4 using a recombinant PLD4-Ig fusion protein as an immunogen, which encodes an amino acid sequence encompassing PLD4 extracellular domain.

[0031]

The present inventors repeated researches in order to acquire an antibody that recognizes PLD4 and revealed that the intended antibody is obtained using a recombinant PLD4-Ig fusion protein as an immunogen, and completed the invention. That is to say, the invention relates to a monoclonal antibody that binds to a PLD4 extracellular domain, or a fragment containing an antigen-binding region thereof.

[0032]

In the invention, PLD4 is a natural molecule that is expressed in human pDC, or an immunologically equivalent molecule to PLD4 that is expressed in human pDC. In the invention, binding of an antibody to PLD4 can be confirmed, for example, as described below.

- Confirmation based on reactivity with human cell:

According to the findings obtained by the present inventors, it is contemplated that PLD4 can be used as a pDC marker, from the fact that it exhibits specific expression in human pDC.

[0033]

Based on such expression profile of PLD4, binding activity with at least partial subset of pDC is one of the important characteristics of the antibody that binds to PLD4 in the invention. The fact that some cell is pDC can be confirmed by a cell surface marker inherent in each cell family. For example, binding to an intended cell is confirmed by double staining of an antibody that binds to a cell surface marker, and an antibody to be confirmed for the binding activity. That is to say, pDC in the invention encompasses cells that express, for example, BDCA2.

[0034]

- Confirmation based on reactivity with transformed cell that expresses PLD4

gene:

The present inventors confirmed that immunological characteristics of PLD4 that is expressed in human pDC is re-constituted when PLD4 gene is expressed under certain conditions. Accordingly, reactivity with PLD4 can be also confirmed based on the reactivity of an antibody for a cell into which a gene that encodes PLD4 is artificially introduced. That is to

say, the invention relates to a monoclonal antibody that binds to a molecule that contains an amino acid sequence that constitutes PLD4 extracellular domain as an extracellular domain, or a fragment containing an antigen-binding region thereof. Meanwhile, the extracellular domain is constituted by an amino acid sequence that corresponds to 54 to 506 from the N terminal in SEQ ID NO 1 (FIG. 1) of the amino acid sequence represented by SEQ ID NO 1.

For example, in a cell that is transformed with an expression vector encompassing DNA that encodes PLD4, immunological characteristics of PLD4 that are expressed in human pDC are maintained. Accordingly, a transformed cell that expresses PLD4 is preferable as a cell for confirming binding property of an antibody to the extracellular domain of PLD4 in the invention. When the reactivity of an antibody by a transformed cell is confirmed in the invention, a non-transformed cell is desirably used as a control.

[0035]

Next, the antibody that binds to PLD4 in the invention may be an antibody that shows cross property with a cell family that is known to express PLD family other than PLD4, or may be an antibody that does not show such cross property. The antibody showing no cross property is preferable as the antibody that binds to PLD4 in the invention. Specifically, the antibody that binds to PLD4 in the invention is preferably an antibody that may not be confirmed for the binding with a cell family that is known to express PLD family other than PLD4, under the same conditions to the conditions where binding to pDC is confirmed.

[0036]

That is to say, the monoclonal antibody that binds to a PLD4 extracellular domain in the invention preferably encompasses a monoclonal antibody that has the immunological characteristics described below.

a) Binding to human pDC,

b) May not be confirmed for binding to one or multiple species of cells selected from a group consisting of monocyte, macrophage, CD34 positive cell, and dendritic cells derived from these cells, under conditions allowing binding to human pDC.

Particularly, the monoclonal antibody of the invention is preferably an antibody that may not be confirmed for binding to monocyte, macrophage, B cell, CD34 positive cell, and dendritic cells derived from these cells, under conditions allowing binding to human pDC.

[0037]

Alternatively, the monoclonal antibody that binds to a PLD4 extracellular domain in the invention preferably encompasses a monoclonal antibody that has immunological characteristics described below.

c) Binding to a transformed cell that is transformed with an expression vector that retains DNA encoding PLD4 in an expressible way,

d) May not be confirmed for binding with the host cell of c) before being transformed, under conditions allowing binding to the transformed cell c).

5 [0038]

The fact that the anti-PLD4 monoclonal antibody is not crossed with other molecules of PLD family in the invention, can be confirmed by employing a cell in which each PLD family was mandatorily expressed. That is to say, mandatory expression is performed by introducing cDNA that encodes the amino acid sequence of each PLD family into an appropriate host cell. The obtained transformed cell is brought into contact with anti-PLD4 monoclonal antibody to be confirmed for the cross property. If binding to a cell that expresses PLD family molecules other than PLD4 is not exhibited, it can be confirmed that the antibody can immunologically distinguish PLD4 from other PLD family molecules. For example, it is confirmed in Examples described below that most of the anti-PLD4 monoclonal antibodies obtained by the invention does not cross with PLD3 and PLD5 having particularly high homology to PLD4, and further PLD1 and PLD2. Accordingly, the monoclonal antibody in the invention is preferably a monoclonal antibody that binds to PLD4, but may not be detected for binding to PLD3, PLD5, PLD1, or PLD2 under the same conditions. If an antibody that can immunologically distinguish these PLD family molecules from PLD4 is used, change of PLD4 expression can be specifically detected.

20 [0039]

Binding of a monoclonal antibody to be confirmed for binding activity, to each species of a cell, can be confirmed, for example, in flow cytometry principle. In order to confirm reactivity of an antibody by flow cytometry principle, it is advantageous to label the antibody with a molecule or atom group that produces a detectable signal. Generally, a fluorescent label or a luminescent label is used. In order to analyze binding of a fluorescent-labeled antibody to a cell by the principle of flow cytometry, a fluorescence-activated cell sorter (FACS) can be used. By using FACS, multiple bindings of an antibody to a cell can be confirmed effectively.

30 [0040]

Specifically, for example, an antibody A that is preliminarily known to be able to identify pDC, and an antibody B to be analyzed for binding characteristics with pDC, are reacted at the same time with a cell family encompassing pDC. The antibody A and the antibody B are on label with a fluorescent signal that can distinguish them from each other. If both of the

signals are detected from the same cell family, it can be confirmed that such antibodies bind to the same cell family. That is to say, it is found out that the antibody A and the antibody B have the same binding characteristics. If the antibody A and the antibody B bind to a different cell family, it is evident that the binding characteristics of them are different from each other.

5 [0041]

Examples of preferable monoclonal antibody in the invention include, for example, monoclonal antibodies produced by hybridomas mp5B7, mp7B4, mp13D4, and mp13H11.

10 The hybridomas mp5B7, mp7B4, mp13D4 and mp13H11 were deposited under Accession Numbers: NITE ABP-1211, NITE ABP-1212, NITE ABP-1213, and NITE ABP-1214 at National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depositary on January 27, 2012. Contents to specify the deposition will be described below.

(1) Name And Address of Deposit Authority

15 Name: National Institute of Technology and Evaluation (NITE) Advanced Industrial Science and Technology Patent Microorganisms Depositary

Address : 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba-ken, 292-0818 JAPAN

(2) Deposit date: January 27, 2012

20 (3) Accession Numbers NITE ABP-1211 (hybridoma mp5B7)
 NITE ABP-1212 (hybridoma mp7B4)
 NITE ABP-1213 (hybridoma mp13D4)
 NITE ABP-1214 (hybridoma mp13H11)

[0042]

25 The monoclonal antibody of the invention may be a fragment containing an antigen-binding region thereof. For example, an antibody fragment encompassing an antigen binding site that is produced by IgG enzymatic digestion, may be used as the antibody in the invention. Specifically, by digestion with papain or pepsin, an antibody fragment such as Fab or F(ab')₂ may be obtained. In addition, an immunoglobulin fragment encompassing a variable region in which complementarily-determining region (CDR) of some monoclonal antibody is transplanted, is encompassed in the fragment containing antigen binding region. It is widely
30 known that these antibody fragments can be used as an antibody molecule that has binding affinity to an antigen. Alternatively, an antibody constructed by gene recombination may be used as long as it maintains the activity necessary for antigen binding. Examples of the antibody constructed by gene recombination include, for example, a chimeric antibody, a CDR transplant antibody, a single chain Fv, a diabody (diabodies), a linear antibody, and a polyspecific

antibody formed by antibody fragments, and the like. A method of obtaining such antibodies based on a monoclonal antibody, or an antibody-producing cell that produces it, is known.

[0043]

The monoclonal antibody of the invention can be obtained by using recombinant
5 PLD4-Ig fusion protein, or a transformed cell that expresses human PLD4 as an immunogen.
That is to say, the present invention relates to a method of preparing a cell that produces a monoclonal antibody that binds to a PLD4 extracellular domain, which contains the processes below.

(1) Process of administering an extrinsic protein containing PLD4 extracellular
10 domain, to an immune animal, and

(2) process of selecting an antibody-producing cell that produces an antibody that binds to PLD4, from antibody-producing cells of the immune animal.

Thus-obtained antibody-producing cell, or immortalized cell of the antibody-producing cell may be cultured, and an intended monoclonal antibody may be collected from the
15 culture. Various methods are known as a method for immortalizing the antibody-producing cell.

[0044]

The transformed cell used as an immunogen in the invention can be obtained, for example, by preparing a cell in which an extrinsic polynucleotide (a) that encodes the amino acid
20 sequence containing the PLD4 extracellular domain described below is retained in an expressible way.

The extrinsic polynucleotide in the present invention refers to a polynucleotide that is artificially introduced into a host cell. In a case where a human cell is used as the cell, a human gene is introduced into the human cell. In such combination, artificially introduced
25 polynucleotide is also referred to as the extrinsic polynucleotide. Accordingly, ectopic expression of PLD4 is encompassed in extrinsic polynucleotide expression.

[0045]

The PLD4 extracellular domain in the invention refers to an amino acid sequence of 54-506 positions that corresponds to the extracellular domain in the amino acid sequence
30 described in SEQ ID NO 1. For example, amino acid sequences containing each of the regions in the order below from the N terminal side are preferable as the amino acid sequence containing the PLD4 extracellular domain in the invention.

[Intracellular region + transmembrane domain + extracellular domain]

Alternatively, an amino acid sequence that is partially lack of the intracellular

region as described below is also encompassed in the amino acid sequence containing the PLD4 extracellular domain in the present invention.

[Partial intracellular region + transmembrane domain + extracellular domain]

Furthermore, a structure that is lack of the intracellular region as described below
5 is encompassed in the amino acid sequence containing the PLD4 extracellular domain in the present invention.

[Transmembrane domain + extracellular domain]

[0046]

Other regions than the extracellular domain in the above-mentioned structure may
10 be a sequence selected from the amino acid sequence represented by SEQ ID NO 1, or may be a combination with another homologous amino acid sequence. For example, an amino acid sequence that constitutes a signal sequence, a transmembrane domain, and an intracellular region can be used as an amino acid sequence of PLD family molecules other than PLD4.
Alternatively, the amino acid sequence of PLD family of other species than human can be also
15 combined. Furthermore, the amino acid sequence that constitutes other regions than the extracellular domain may contain mutation within a range where each of the functions of the regions can be maintained. In addition, other regions can be interposed between each of the regions. For example, between the signal sequence and the extracellular domain, an epitope tag such as FLAG can be also inserted. Particularly, the signal sequence is a region that is
20 subjected to processing in the step of transport to the cell membrane surface after translation of the protein, and is removed. Accordingly, an arbitrary amino acid sequence that induces passage of the translated protein through the cell membrane can be used as a signal sequence. More specifically, an amino acid sequence of PLD4 (SEQ ID NO 1) is preferable as an amino acid sequence containing the PLD4 extracellular domain.

25 [0047]

Accordingly, the above-mentioned polynucleotide (a) in the invention may be an arbitrary base sequence that encodes the amino acid sequence that constitutes the above-mentioned structure [Intracellular region + transmembrane domain + extracellular domain].
For example, the amino acid sequence of SEQ ID NO 1 is encoded by the cDNA base sequence
30 described in SEQ ID NO 44.

[0048]

The recombinant PLD4-Ig fusion protein as an immunogen in the invention may be obtained by introducing an expression vector which retains the aforementioned polynucleotide in an expressible way, into an appropriate host cell. The cDNA base sequence

of the recombinant PLD4-Ig fusion protein is represented by SEQ ID NO 125, and the amino acid sequence is represented by SEQ ID NO 126.

[0049]

The host cell in the invention is preferably a mammalian cell. Specifically, a cell
5 derived from a human, a monkey, a mouse, or a rat may be used as the host cell. Particularly, human-derived cell is preferable as the host cell. For example, HEK-293T cell is a human embryo-derived renal cell strain that may be preferably used as the host cell in the invention. The HEK-293T cell is available as ATCC CRL-11268. In addition to that, a cell derived from an immune animal may be used as the host cell. If a cell derived from an immune animal is
10 used as an immunogen, immune response against the host cell is small. Therefore, an antibody against the PLD4 extracellular domain, which is expressed extrinsically, can be obtained effectively. Accordingly, for example, when a mouse is used as the immune animal, a cell derived from the mouse can be also used as the host cell.

[0050]

15 The above-mentioned polynucleotide can be loaded to a vector that can induce expression in a host cell to transform the cell. A commercially available vector that can induce expression in a mammalian cell may be used. For example, an expression vector such as pCMV-Script(R) Vector, pSG5 Vector (manufactured by Stratagene), pcDNA3.1 (manufactured by Invitrogen), pMXs-IP retroviral vector (manufactured by Cell BioLabs), and the like can be
20 used for the invention.

[0051]

Thus-obtained transformed cell is administered to an immune animal, with an additional component such as an adjuvant if necessary. As the adjuvant, Freund's complete adjuvant and the like may be used. In a case where a mouse is used as the immune animal,
25 purified recombinant PLD4-Ig fusion protein is administered to BALB/c mouse. As the adjuvant, Freund's Adjuvant, Complete and Incomplete (manufactured by SIGMA) were used, and administered in 200 µg/mouse at the first time, and 50 µg/mouse at the second time to fourth time. Generally, an immunogen is administered multiple times intervally until the antibody titer increases. For example, in a case of short time immunization method, a transformed cell is
30 administered at an interval of 2 to 4 days, more specifically 3 days, and after 2 to 3 times of the administration, antibody-producing cells can be collected. In addition, the antibody-producing cells can be also collected after the administration 5 to 6 times at an interval of once or so a week.

[0052]

In order to obtain the monoclonal antibody in the invention, the collected antibody-producing cell is cloned. For the cloning, the antibody-producing cell is preferably immortalized. For example, a cell fusion method represented by hybridoma method, or transformation by Epstein-Barr virus (EBV) may be used as a method for immortalizing the antibody-producing cell.

[0053]

An antibody-producing cell produces one kind of antibody per one cell. Accordingly, if a cell population derived from one cell can be established (that is to say, cloning), a monoclonal antibody can be obtained. The hybridoma method refers to a method in which an antibody-producing cell is fused with an appropriate cell strain, immortalized, and then cloned. The immortalized antibody-producing cell can be cloned by a method such as limiting dilution method. Many cell strains that are useful in the hybridoma method are known. These cell strains are excellent in immortalization efficiency of a lymphocyte-based cell, and have various gene markers that are necessary for selecting cells that have succeeded in the cell fusion. Furthermore, in a case where acquisition of the antibody-producing cell is intended, a cell strain that is lack of antibody production ability may be also used.

[0054]

For example, mouse myeloma P3x63Ag8.653 (ATCC CRL-1580) or P3x63Ag8U.1 (ATCC CRL-1597) is universally used as a useful cell strain for a cell fusion method of a mouse or a rat. Generally, a hybridoma is prepared by fusion of homogeneous cells. However, a monoclonal antibody may be also acquired from a heterohybridoma between close heterogeneous species.

[0055]

A specific protocol of the cell fusion is known. That is to say, an antibody-producing cell of an immune animal is mixed with an appropriate fusion partner, and subjected to cell fusion. As the antibody-producing cell, a splenic cell, a lymphocyte cell collected from the lymph node, peripheral blood B cell, or the like may be used. As the fusion partner, various cell strains described above may be used. For the cell fusion, a polyethylene glycol method or an electric fusion method is used.

Next, cells that have succeeded in the cell fusion are selected based on a selection marker possessed by the fusion cell. For example, in a case where HAT sensitive cell strain is used in the cell fusion, cells that have succeeded in the cell fusion are selected by selecting cells that grow in the HAT medium. Furthermore, an antibody produced by the selected cells is confirmed whether it has intended reactivity.

[0056]

Each of the hybridoma is screened based on the reactivity of the antibody. That is to say, a hybridoma that produces an antibody that binds to PLD4 is selected by the method as described above. Preferably, in a case where selected hybridoma is subcloned, and production
5 of intended antibody is finally confirmed, the hybridoma is selected as a hybridoma that produces the monoclonal antibody of the invention.

[0057]

Specifically, intended hybridoma can be selected based on reactivity with a human cell, or reactivity with a transformed cell that expresses PLD4 gene. An antibody that
10 binds to a cell can be detected on the principle of an immunoassay. For example, ELISA, in which a cell is used as an antigen, can be used in detection of intended antibody. Specifically, a culture supernatant of the hybridoma is brought into contact with a carrier that immobilizes human pDC, or a transformed cell used as an immunogen. In a case where the culture supernatant contains the intended antibody, the antibody is captured by the cell immobilized on
15 the carrier. Then, the solid phase is isolated from the culture supernatant, washed if necessary, and then the antibody captured on the solid phase can be detected. In detection of the antibody, an antibody that recognizes the antibody may be used. For example, an antibody of a mouse can be detected by anti-mouse immunoglobulin antibody. If the antibody that recognizes an antibody is on label, the detection thereof is ease. As the label, an enzyme, a fluorescent
20 pigment, a luminescent pigment, or the like may be used.

On the other hand, as the carrier that immobilizes the cell, a particle, or the internal wall of a microtiter plate may be used. The surface of a particle or a container made of plastic, can immobilize the cell by physical adsorption. For example, beads or a reaction container made of polystyrene may be used as a carrier for immobilizing the cell.

25 [0058]

In selecting the hybridoma, there may be a case where predicted is not production of an antibody against PLD4, but production of an antibody against a host cell of a transformed cell used as an immunogen. For example, when a human cell is used as an immunogen and a mouse is used as an immune animal as shown in Examples, the human cell is recognized as a
30 foreign substance, and it is predicted that an antibody that binds to it is produced. In the invention, acquisition of an antibody that recognizes PLD4 is intended. Accordingly, acquisition of an antibody that recognizes other human cell antigens than PLD4 is not necessary. In order to exclude a hybridoma that produces such antibody by screening, an antibody not intended may be preliminarily absorbed before confirmation of the antibody reactivity.

[0059]

Antibodies that are not intended can be absorbed by an antigen that binds to an antibody predicted to exist. Specifically, for example, an antibody against other human cell antigens other than PLD4 can be absorbed by a cell for which expression of PLD4 may not be
5 detected. In the invention, the host cell used for the immunogen is preferable as an antigen for absorbing antibodies not intended.

[0060]

A monoclonal antibody confirmed to have binding activity to an antigen, is confirmed for practical influence on the activity of pDC, if necessary. The influence on pDC
10 can be confirmed, for example, by a method described below.

[0061]

The monoclonal antibody of the invention can be collected from a culture that is obtained by culturing a hybridoma that produces the monoclonal antibody. The hybridoma may be cultured in vitro or in vivo. In the culture in vitro, the hybridoma may be cultured using a
15 known medium such as RPMI 1640. Immunoglobulins secreted by the hybridoma are accumulated in the culture supernatant. Accordingly, the monoclonal antibody of the invention may be obtained by collecting the culture supernatant, and purifying it if necessary. Purification of immunoglobulin is ease in a medium that is not added with the serum. However, for the purpose of rapid growth of the hybridoma and promotion of antibody
20 production, 10% or so of bovine fetal serum may be also added to the medium.

[0062]

The hybridoma may be also cultured in vivo. Specifically, the hybridoma may be cultured in the abdominal cavity by inoculating the hybridoma into the abdominal cavity of a nude mouse. The monoclonal antibody is accumulated in the ascites. Accordingly, ascites is
25 collected, and purified if necessary, to obtain necessary monoclonal antibody. The obtained monoclonal antibody may be suitably modified, or processed depending on the purpose.

[0063]

The monoclonal antibody of the invention can be expressed by acquiring cDNA that encodes the antigen binding region of the antibody from the hybridoma, and inserting it into
30 an appropriate expression vector. A technology for acquiring cDNA that encodes a variable region of an antibody and express it in an appropriate host cell is known. In addition, an approach is known in which a variable region containing an antigen binding region is bound to a constant region to give a chimeric antibody. For example, a gene of a variable region may give a chimeric antibody by binding to genes that encode a human IgG1 heavy chain constant region

and human Ig kappa light chain constant region, respectively. Furthermore, it is known that antigen binding activity of a monoclonal antibody can be transplanted to another immunoglobulin by incorporating CDR that constitutes a variable region into a frame region of another immunoglobulin molecule. Using this, a method is established of transplanting antigen binding activity possessed by a heterogeneous immunoglobulin to a human immunoglobulin. For example, there may be a case where a partial amino acid sequence of a frame region supporting CDR is transplanted to a human variable region from a variable region of a mouse antibody. Next, these humanized, re-constituted variable regions of a human antibody may be linked to a constant region of a human antibody, to obtain a humanized antibody.

10 [0064]

Examples of preferable monoclonal antibody in the invention include, for example, monoclonal antibodies produced by hybridomas mp5B7, mp7B4, mp13D4, mp13H11, and the like, which have been deposited under Accession Numbers: NITE ABP-1211, NITE ABP-1212, NITE ABP-1213, and NITE ABP-1214, respectively.

15 As the chimeric antibody containing the variable region, or the humanized antibody in which CDR that constitutes the variable region is transplanted, an antibody having a constant region derived from IgG or IgM is contained preferably in the antibody of the invention. Particularly, the antibody of the invention is more preferably an antibody that has a combination of:

20 heavy chain CDR1: DYNLH,
 CDR2: YIYPYNGNTGYNQKFKR,
 CDR3: GGIYDDYYDYAIDY and
 light chain CDR1: RASENIYSHIA,
 CDR2: GATNLAH,
 25 CDR3: QHFWGTP,

as a sequence of CDR that constitutes the variable region of the antibody, an antibody that has a combination of:

heavy chain CDR1: SHYYWT,
 CDR2: YISYDGSNNYNPSLKN,
 30 CDR3: EGPLYYGNPYWYFDV and
 light chain CDR1: RASQDIDNYLN,
 CDR2: YTSRLHS,
 CDR3: QQFNTLP,

as a sequence of CDR that constitutes the variable region, or an antibody that has a combination

of:

heavy chain CDR1: SHYYWS,
 CDR2: YISYDGSNNYNPSLKN,
 CDR3: EGPLYYGNPYWYFDV and

5 light chain CDR1: RASQDIDNYLN,
 CDR2: YTSRLHS,
 CDR3: QQFNTLP,

as a sequence of CDR that constitutes the variable region.

The present inventors have confirmed that the monoclonal antibody against PLD4
 10 has CDC action for a PLD4 expression cell. Accordingly, the antibody that has IgG- or IgM-
 derived constant region, has an action of cytotoxicity for PLD4 expression cell by CDC action.
 Such antibody is useful for suppressing the cell number of a PLD4 expression cell such as pDC.

A chimeric antibody, or a humanized antibody that recognizes PLD4 can be
 prepared by genetic engineering using a polynucleotide that encodes them.

15 [0065]

An antibody that can specifically recognize PLD4 was not obtained. An
 antibody that recognizes PLD4 has been provided for the first time by the immunogen of the
 invention. That is to say, the invention provides an antibody that recognizes PLD4, which can
 be obtained by the processes described below.

20 (1) Process of administering a protein containing a PLD4 extracellular domain, to
 an immune animal,

(2) process of selecting an antibody-producing cell that produces an antibody that
 binds to PLD4, from antibody-producing cells of the immune animal, and

(3) process of culturing the antibody-producing cell selected in (2), and collecting
 25 an antibody that recognizes PLD4 from the culture.

[0066]

It has been revealed that PLD4 is specifically expressed in human pDC. The
 specific expression in human pDC was also confirmed by gene expression analysis with SAGE
 by the present inventors. However, PLD4 expression level was all analyzed based on mRNA in
 30 a report of the past. In addition, the PLD4 protein is known to be expressed only in the
 cytoplasm. It has been revealed by the invention that PLD4 is also expressed on the cell
 surface. Since an antibody that can detect PLD4 was not provided, analysis for the state of
 protein expression was not conventionally performed. The antibody that binds to a PLD4
 extracellular domain provided by the invention has realized analysis of the PLD4 protein.

[0067]

As confirmed practically by the present inventors, the monoclonal antibody that binds to a PLD4 extracellular domain based on the invention has specifically detected human pDC. That is to say, the invention relates to a method of detecting a plasmacytoid dendritic
5 cell, which contains steps of bringing a monoclonal antibody that binds to a PLD4 extracellular domain, or a fragment containing an antigen-binding region thereof into contact with a test cell, and detecting a monoclonal antibody that binds to the cell, or a fragment containing an antigen-binding region thereof.

[0068]

10 By detection of PLD4 based on the invention, it can be confirmed whether some cell is pDC or not. That is to say, the invention provides a method of identifying pDC using PLD4 as an index. Alternatively, a cell for which PLD4 is detected may be isolated based on the invention, whereby to isolate human pDC. That is to say, the invention provides a method of isolating pDC using PLD4 as an index.

15 [0069]

In the invention, a monoclonal antibody that binds to a PLD4 extracellular domain, or a fragment containing an antigen-binding region thereof may be on label. For example, the antibody can be easily detected by labeling it with a luminescent pigment or fluorescent pigment. More specifically, fluorescent pigment-labeled antibody is brought into
20 contact with a cell population that possibly contains pDC, and a cell that binds to the antibody of the invention can be detected using the fluorescent pigment as an index. Furthermore, if a cell detected with the fluorescent pigment is isolated, pDC can be isolated. A series of steps can be easily implemented in the principle of FACS.

[0070]

25 Alternatively, the antibody of the invention may be also as bound to a solid phase carrier such as a magnetic particle. The antibody that is bound to the solid phase carrier recognizes PLD4, and pDC is captured on the solid phase carrier. As a result thereof, pDC can be detected or isolated.

[0071]

30 The antibody that is necessary in the pDC detection method based on the invention may be supplied as a reagent for detection of pDC. That is to say, the invention provides a reagent for detection of pDC containing a monoclonal antibody that binds to a PLD4 extracellular domain, or a fragment containing an antigen-binding region thereof. In the reagent for detection of pDC of the invention, a positive control, or a negative control may be

combined in addition to the antibody. For example, a transformed cell that expresses PLD4 extracellular domain used as an immunogen, or pDC collected from a human, or the like may be used as a positive control. Usually, only little human pDC can be obtained from the peripheral blood. Accordingly, a transformed cell is particularly preferable as a positive control in the reagent of the invention. On the other hand, as the negative control, an arbitrary cell that does not express PLD4 may be used.

[0072]

That is to say, the invention provides a kit for detection of human pDC containing a monoclonal antibody that binds to a PLD4 extracellular domain, or a fragment containing an antigen-binding region thereof.

[0073]

Furthermore, the inventors analyzed the influence of the antibody that binds to a PLD4 extracellular domain on pDC. As a result thereof, it was confirmed that the antibody that binds to a PLD4 extracellular domain suppresses pDC activity. That is to say, the invention relates to a method of suppressing the activity of an interferon-producing cell, which contains a step of bringing any one of the components below into contact with pDC:

(a) a monoclonal antibody that binds to PLD4 and suppresses pDC activity, or a fragment containing an antigen-binding region thereof, and

(b) an immunoglobulin in which a complementarity-determining region of the monoclonal antibody (a) is transplanted, or a fragment containing an antigen-binding region thereof.

[0074]

Alternatively, the invention relates to a method of suppressing pDC activity in a living organism, which contains a step of administering any one of the components below to a living organism:

(a) a monoclonal antibody that binds to PLD4 and suppresses pDC activity, or a fragment containing an antigen-binding region thereof,

(b) an immunoglobulin in which a complementarity-determining region of the monoclonal antibody (a) is transplanted, or a fragment containing an antigen-binding region thereof, and

(c) a polynucleotide that encodes the component described in (a) or (b).

[0075]

The pDC in the invention refers to a cell that has IFN production ability, and expresses PLD4 on the cell surface. Hereinafter, unless otherwise stated particularly, pDC is

not only a cell that is a precursor cell of a dendritic cell, but contains a cell that has IFN production ability, and expresses PLD4 on the cell surface. A method of identifying such pDC is known. For example, pDC can be distinguished from other blood cells using several cell surface markers as an index. Specifically, the profile of a human pDC cell surface marker is as described below (Shortman, K. and Liu, YJ. Nature Reviews 2: 151-161, 2002). In recent years, there is also a report which positioned BDCA-2 positive cell as pDC (Dzionek, A. et al. J. Immunol. 165: 6037-6046, 2000).

[Profile of cell surface antigen of human pDC]

CD4 positive and CD123 positive,

Lineage (CD3, CD14, CD16, CD19, CD20, and CD56) negative, CD11c negative

Accordingly, a cell that has the expression profile of these known markers, and has IFN production ability may be also referred to as pDC. Furthermore, a cell that belongs to a cell family having a different profile from the expression pattern of the expression profile of these markers, but having IFN production ability in a living organism is encompassed in pDC.

Furthermore, examples of common characteristics exhibited by human pDC include the characteristics as described below.

[Morphological Characteristic of Cell]

- Similar to plasma cell
- Round cell having smooth cell surface
- Relatively large nucleus

[Functional Characteristic of Cell]

• Production of a large amount of type I IFN in a short time at the time of virus infection

- Differentiation into dendritic cell after virus infection

[0076]

Suppression of the pDC activity in the invention refers to suppression of at least one of the functions of pDC. Examples of the pDC functions include IFN production and cell survival. The cell survival can be referred to as the cell number in other words. Accordingly, suppression of either one or both of these functions is referred to as the suppression of pDC activity. It has been revealed that type I IFN produced by pDC is a cause for various diseases. Accordingly, suppression of the pDC cell number or IFN production is useful as a strategy for treating such diseases.

For example, the relation of pathological conditions of an autoimmune disease with IFN α has been pointed out. Most of IFN α is produced by pDC. Accordingly, if the

production thereof is suppressed, pathological conditions caused by IFN α can be alleviated. Meanwhile, in the invention, suppression of IFN production by pDC refers to suppression of production of at least one kind of IFN produced by pDC. The IFN in the invention is preferably type I IFN. Among them, IFN α is important.

5 [0077]

That is to say, the invention relates to a suppressor for IFN production, which contains the antibody that binds to a PLD4 extracellular domain as an active component. Alternatively, the invention provides a method of suppressing IFN production, which contains a process of administering an antibody that binds to a PLD4 extracellular domain. Furthermore,
10 the invention relates to use of an antibody that binds to a PLD4 extracellular domain in preparation of a pharmaceutical composition that suppresses IFN production.

[0078]

A cell that produces a large amount of IFN in a small number of the cells is encompassed in the pDC. For example, a precursor cell of a dendritic cell that is received with
15 stimulation by a virus or the like produces most of IFN produced by a living organism. Suppression of the cell number of pDC that produces a large amount of IFN leads to suppression of IFN production as results. Accordingly, suppression of the pDC cell number can also alleviate pathological conditions caused by IFN α .

In a preferred aspect of the invention, it was confirmed that the anti-PLD4
20 monoclonal antibody binds to a PLD4 expression cell, and imparts cytotoxicity by Complement Dependent Cytotoxicity (CDC) action. The CDC action is one of the important action mechanisms of an antibody drug. The anti-PLD4 monoclonal antibody of the invention also has potent cytotoxicity action for a PLD4 expression cell such as pDC by the CDC action. That is to say, the anti-PLD4 monoclonal antibody in a preferred aspect, can be expected for effects of
25 suppressing IFN production not only by a mechanism of suppressing IFN production, but also by cytotoxicity for pDC.

[0079]

The antibody that recognizes a PLD4 extracellular domain used in the invention can be obtained based on the method as described above. The antibody in the invention may be
30 any class. In addition, a biological species from which the antibody is derived is not limited. Furthermore, a fragment containing a antigen binding region of the antibody can be used as the antibody. For example, an antibody fragment containing an antigen binding site produced by enzymatic digestion of IgG may be used as the antibody in the invention. Specifically, an antibody fragment such as Fab or F(ab')₂ may be obtained by digestion by papain or pepsin. It

is widely known that these antibody fragments can be used as an antibody molecule that has binding affinity to an antigen. Alternatively, an antibody constructed by gene recombination may be also used as long as it maintains necessary antigen binding activity. Examples of the antibody constructed by gene recombination include a chimeric antibody, a CDR transplantation antibody, a single chain Fv, a diabody (diabodies), a linear antibody, and a polyspecific antibody formed by an antibody fragment, and the like. A method of obtaining these antibodies based on a monoclonal antibody is known.

[0080]

The antibody in the invention may be modified, if necessary. According to the invention, the antibody that recognizes a PLD4 extracellular domain has an action of suppressing pDC activity. That is to say, a possibility has been contemplated that the antibody itself has cytotoxicity for pDC. A subclass of the antibody exhibiting strong effector action is known. Alternatively, effects of suppressing pDC activity can be further increased by modifying the antibody with a cytotoxic agent. Examples of the cytotoxic agent include the agents described below.

Toxins: Pseudomonas Endotoxin (PE), diphtheria toxin, and ricin

Radioactive isotope: Tc99m, Sr89, I131, and Y90

Anti-cancer agent: calicheamicin, mitomycin, and paclitaxel

Toxins containing a protein can binds to an antibody, a fragment thereof, or the like by a bifunctional reagent. Alternatively, a gene that encodes the toxins may be also conjugated with a gene that encodes the antibody, to give a fusion protein of them. A method of binding a radioactive isotope to an antibody is also known. For example, a method of labeling an antibody with a radioactive isotope using a chelate agent is known. Furthermore, an anti-cancer agent can bind to an antibody by using a sugar chain, a bifunctional reagent, or the like.

[0081]

In the invention, artificially structure-modified antibody may be also used as an active component. For example, various modifications are known to improve cytotoxicity or stability of an antibody. Specifically, an immunoglobulin is known, in which the sugar chain of the heavy chain is modified (Shinkawa, T. et al. J. Biol. Chem. 278: 3466-3473. 2003). Modification of the sugar chain increased Antibody Dependent Cell-mediated Cytotoxicity (ADCC) activity of the immunoglobulin.

[0082]

The antibody that binds to a PLD4 extracellular domain suppresses the activity of

pDC when the antibody is brought into contact with the pDC. Accordingly, such antibody can be used as an agent for suppressing pDC activity, or in a method of suppressing pDC. That is to say, the invention provides an agent for suppressing pDC activity, which contains at least one kind of a component selected from a group consisting of (a) to (c) described below as an active component. Alternatively, the invention relates to a method of suppressing pDC activity, which contains a step of administering at least one kind of a component selected from a group consisting of (a) to (c) described below. Furthermore, the invention relates to use of at least one kind of a component selected from a group

consisting of (a) to (c) described below in preparation of an agent for regulating pDC activity.

(a) Antibody that binds to a PLD4 extracellular domain, or a fragment containing an antigen-binding region thereof,

(b) an immunoglobulin in which a complementarity-determining region of the monoclonal antibody (a) is transplanted, or a fragment containing an antigen-binding region thereof.

As the monoclonal antibody that suppresses pDC activity in the invention, a monoclonal antibody that recognizes a PLD4 extracellular domain may be used. One kind or multiple kinds of the monoclonal antibodies may be used in the invention. For example, multiple species of the monoclonal antibodies that recognize a PLD4 extracellular domain may be blended, and used in the invention.

[0083]

The fact that the antibody has an action of suppressing IFN production activity of pDC, can be confirmed in the manner as described below. pDC produces a large amount of IFN upon viral stimulation. The antibody is given to pDC before, after, or at the same time of viral stimulation, and IFN production ability is compared with a control in which the antibody is not given to pDC. The IFN production ability can be evaluated by measuring IFN- α or IFN- β contained in the supernatant of a pDC culture. As a result of the comparison, if the amount of IFN in the supernatant is significantly lowered by addition of the antibody, it can be confirmed that the tested antibody has an action of suppressing the IFN production ability. A method of measuring these IFNs is known. pDC is a cell that produces most of IFN in a living organism. Accordingly, suppression of IFN production ability of pDC can regulate IFN production state in a living organism.

[0084]

The activity of pDC in the invention encompasses maintenance of the pDC cell number. Accordingly, suppression of pDC activity in the invention encompasses suppression of

the pDC cell number. If the pDC cell number is confirmed to be suppressed in the presence of the antibody, the antibody is found out to suppress the pDC activity. Similarly to the IFN production, an inactive immunoglobulin that is derived from the same animal species as the species of the antibody to be confirmed for the activity, may be used as a control for comparison.

5 The pDC cell number can be compared quantitatively by cell counting. The cell number can be counted with FACS or a microscope.

[0085]

Furthermore, pDC is also assumed to be differentiated into a cell that induces Th2 called Dendritic Cell 2 (DC2) as a result of infection by virus and the like. If pDC of IFN
10 production upon virus stimulation is suppressed, there is a possibility that differentiation to Th2 can be also suppressed. Accordingly, the monoclonal antibody of the invention that suppresses IFN production can be also expected to have therapeutic effects for various allergy diseases.

[0086]

In a case where the antibody that recognizes a PLD4 extracellular domain is
15 administered to a host that is different from the biological species from which the antibody is derived, it is desirable to process the antibody into a form that is hardly recognized as a foreign substance by the host. For example, immunoglobulin may be rendered to be hardly recognized as a foreign substance by processing the molecule as described below. A method of processing an immunoglobulin molecule as described below is known.

20 • Fragment containing antigen binding region, from which the constant region is deleted (Monoclonal Antibodies: Principles and Practice, third edition, Academic Press Limited. 1995; Antibody Engineering, A Practical Approach, IRL PRESS, 1996)

• Chimeric antibody constituted with an antigen binding region of a monoclonal antibody and a constant region of host immunoglobulin (Gene Expression Experiment Manual,
25 Kodansha Ltd. 1994 (edited by Isao ISHIDA and Tamie ANDO))

• CDR-substituted antibody in which the complementarity-determining region (CDR) in a host immunoglobulin is substituted with CDR of a monoclonal antibody (Gene Expression Experiment Manual, Kodansha Ltd. 1994 (edited by Isao ISHIDA and Tamie ANDO))

30 [0087]

Alternatively, the gene of a human immunoglobulin variable region can be also acquired by phage display method (McCafferty J et al., Nature 348:552-554, 1990; Kretzschmar T et. al., Curr Opin Biotechnol. 2002 Dec; 13(6): 598-602.). In the phage display method, the gene that encodes the human immunoglobulin variable region is incorporated into the phage

gene. Using various immunoglobulin genes as a source, a phage library can be also prepared. A phage expresses the variable region as a fusion protein of the protein that constitutes itself. The variable region expressed on the phage surface by the phage maintains binding activity to antigen. Accordingly, selection of a phage that binds to an antigen or a cell in which the antigen is expressed, or the like, can screen a phage in which a variable region having intended binding activity is expressed from a phage library. Furthermore, a particle of thus-selected phage retains a gene that encodes the variable region having intended binding activity. That is to say, a gene that encodes a variable region having intended binding activity can be acquired using the binding activity of the variable region as an index in the phage display method.

10 [0088]

The antibody that recognizes a PLD4 extracellular domain, or an antibody fragment containing at least antigen binding region thereof in the agent for suppressing pDC activity, or the method of suppressing pDC activity according to the invention, can be administered as a protein, or a polynucleotide that encodes the protein. In administering the polynucleotide, it is desirable to use a vector in which a polynucleotide that encodes intended protein, is disposed under control of an appropriate promoter so as to express the intended protein. In the vector, an enhancer or a terminator may be also disposed. A vector is known that retains genes of a heavy chain and a light chain that constitute an immunoglobulin, and can express the immunoglobulin molecule. The vector that can express an immunoglobulin can be administered by being introduced into a cell. In administration to a living organism, a vector that can infect a cell by being administered into a living organism, can be administered as it is. Alternatively, a vector may be also first introduced into a lymphocyte isolated from a living organism, and returned again into the living organism (ex vivo).

[0089]

25 The amount of the monoclonal antibody that is administered to a living organism in the agent for suppressing pDC activity, or the method of suppressing pDC activity based on the invention, is usually 0.5 mg to 100 mg, for example 1 mg to 50 mg, preferably 2 mg to 10 mg per 1 kg of the body weight as an immunoglobulin. The administration interval of the antibody to a living organism can be suitably regulated such that an effective concentration of the immunoglobulin in the organism can be maintained during the treatment period. Specifically, administration may be performed, for example, at an interval of 1 to 2 weeks. The administration route is arbitrary. One of ordinary skill in the art can suitably select an effective administration route in the treatment. Specifically, examples of the administration route include oral or non-oral administration. For example, the antibody can be administered systemically or

30

locally by intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection, or the like. Examples of an appropriate formulation for non-oral administration in the invention include an injection, a suppository, a spray, and the like. In addition, in a case where an immunoglobulin is administered to a cell, the immunoglobulin is administered to a culture liquid in usually 1 $\mu\text{g/mL}$, preferably 10 $\mu\text{g/mL}$ or more, more preferably 50 $\mu\text{g/mL}$ or more, and further preferably 0.5 mg/mL or more.

[0090]

In the agent for suppressing pDC activity or the method of suppressing pDC activity of the invention, the monoclonal antibody may be administered by any method to a living organism. Usually, the monoclonal antibody is blended with a pharmaceutically acceptable carrier. Along with the monoclonal antibody, additives such as a thickening agent, a stabilizer, a preservative, and a solubilizing agent may be blended if necessary. Examples of such carrier or additives include lactose, citric acid, stearic acid, magnesium stearate, sucrose, starch, talc, gelatin, agar, vegetable oil, ethylene glycol, and the like. The terms called "pharmaceutically acceptable" refers to those approved by government supervisory of each country, or those listed in a pharmacopoeia of each country or a pharmacopoeia generally acknowledged for use in an animal, a mammalian animal, and particularly human. The agent for suppressing pDC activity of the invention may be also supplied in a form of a lyophilized powder or tablet in a single dose or multiple doses. The lyophilized powder or tablet may be further combined with sterilized water, physiological saline, or a buffer solution for injection for dissolving the composition to a desired concentration before administration.

[0091]

Furthermore, in a case where the agent for suppressing pDC activity of the invention is administered as an immunoglobulin expression vector, the heavy chain and the light chain are co-transfected with a separate plasmid, and each of the plasmids can be administered in 0.1 to 10 mg, for example, 1 to 5 mg per 1 kg of the body weight. In addition, a vector of 1 to 5 $\mu\text{g}/10^6$ cells is used for introduction into a cell in vitro. Hereinafter, the invention will be further specifically explained based on Examples.

Meanwhile, the entire prior art documents cited in the present specification are incorporated into the present specification by reference.

Although the invention will be further specifically described with Examples below, the invention is not limited to such Examples at all.

Examples

[0092]

Example 1

A. Analysis of PLD4 expression

A-1) Analysis using SAGE library

5 Gene expressions in resting pDC, human monocyte, and activated pDCs treated with inactive herpes virus (HSV-1), were compared by SAGETM (Serial Analysis of Gene Expression) method. The analysis method is as described below.

From the human peripheral blood, the monocyte was isolated as CD14 positive cell and the human pDC cell was isolated as BDCA-4 positive cell with BDCA-4+ isolation kit
 10 (Miltenyi Biotec company) and MACS system (Miltenyi Biotec company). Furthermore, the human pDC cell was cultured for 12 hours in the presence of inactive HSV-1, whereby to prepare activated pDC (pDC+HSV). Total RNA was extracted from each of the cells, and the SAGE library was prepared using I-SAGETM kit (Invitrogen company). The obtained data of about 100,000 tagged base sequences were analyzed with SAGE2000 Analysis Software
 15 (Invitrogen company). As a result thereof, the score value of monocyte/pDC/pDC+HSV was 0/9/0 gene, that is to say, PLD4 (Phospholipase D family, member 4, C14orf175; GenBank Accession Number: NM_138790.2) was found, which is a known gene as a gene exhibiting resting pDC cell specific expression (FIG. 1, Tao et al., Nat. Methods 2(8), 591-598(2005); Clark et al., Genome Res. 13(10), 2265-2270(2003)). PLD4 is a 506 amino acid sequence (SEQ ID
 20 NO: 1) encoded by a base sequence represented by SEQ ID NO: 44. The PLD4 protein has two tentative PDE regions (Phosphodiesterase motif) constituted with two HKD motifs (His-x-Lys-x-x-x-x-Asp amino acid sequence, the x is the other amino acids) conserved in the C terminal region, and a tentative phosphorylation site (Thr 472). The structure of the PLD4 protein is predicted as type II monotropic transmembrane protein. In addition, the PLD4 protein does not
 25 have PX region (Phox homology domain) and PH region (Pleckstrin homology domain) in the N terminal region, which are possessed by PLD1 and PLD2 that are of classical PLD family (FIGFIGS. 1 and 2).

[0093]

A-2) Expression analysis of PLD4 mRNA in human various cells responsible for
 30 immunity by quantitative real time PCR

Expression of PLD4 mRNA in a blood cell was specifically reviewed. From the human peripheral blood, each of the cells was isolated and taken by a cell sorter. From each of the cell family isolated and taken, RNA was extracted, and cDNA was synthesized. Using the obtained cDNA as a template, quantitative real time PCR was performed, and expression level of

PLD4 mRNA was analyzed.

For the quantitative real time PCR reaction, quantitative PCR was performed with ABI PRISM 7000 using Platinum SYBR Green qPCR Super Mix-UDG kit (Invitrogen company). Sequence Detection System Software (Applied Biosystem company) was used in the data analysis. The PCR reaction conditions and the base sequence of the used primers are as follows.

Forward primer for PLD4: 5' ATG GAC TGG CGG TCT CTG 3' (SEQ ID NO: 45)

Reverse primer for PLD4: 5' TGG AAG GTC TTC TCC AGG TC 3' (SEQ ID NO: 46)

Forward primer for GAPDH: 5' AGC CAC ATC GCT CAG ACA C 3' (SEQ ID NO: 47)

Reverse primer for GAPDH: 5' GCC CAA TAC GAC CAA ATC C 3' (SEQ ID NO: 48)

1 cycle at 58°C for 2 minutes,

1 cycle at 95°C for 10 minutes,

50 cycles of [95°C for 15 second, and 60°C for 60 seconds],

pDC, pDC (pDC+HSV) stimulated with HSV, B cell (CD19+ cell), activated B cell (CD19+ cell), T cell (CD3+ cell), and activated T cell stimulated with Inomycin and PMA (Phorbol 12-myristate 13-acetate) were reviewed. As a result, it was illustrated that PLD4 expression was specifically high in the pDC at the resting stage, and low in the CD19⁺ B cell. The other human blood fraction cDNA used BDTM MTC Multiple Tissue cDNA Panels (Cat. No. 636750, Takara Bio company) (FIG. 5).

[0094]

A-3) Expression analysis of PLD4 mRNA in human tissue by quantitative real time PCR

Furthermore, expressions in other organs or tissues were reviewed by quantitative PCR using ABI PRISM 7000 (Applied Biosystem company). As the cDNA panel, BDTM MTC multiple tissue cDNA panel (Human I; Cat. No. 636742, Human immune; Cat. No. 636748; all Takara Bio company) was used. The base sequences of the used primers are represented as follows.

Forward primer for PLD4: 5' ATG GAC TGG CGG TCT CTG 3' (SEQ ID NO: 49)

Reverse primer for PLD4: 5' TGG AAG GTC TTC TCC AGG TC 3' (SEQ ID

NO: 50)

Forward primer for GAPDH: 5' AGC CAC ATC GCT CAG ACA C 3' (SEQ ID

NO: 51)

Reverse primer for GAPDH: 5' GCC CAA TAC GAC CAA ATC C 3' (SEQ ID

5 NO: 52)

The quantitative PCR was performed with ABI PRISM 7000 using Platinum SYBR Green qPCR Super Mix-UDG kit (Invitrogen company). Sequence Detection System Software (Applied Biosystem company) was used in the analysis. The reaction conditions are as described below.

10 Step 1: 1 cycle at 50°C for 2 minutes

Step 2: 1 cycle at 95°C for 10 minutes

Step 3: 50 cycles at 95°C for 15 seconds and 60°C for 1 minute

Expressions of PLD4 genes were compared between each of the tissues by standardization with gene expression level of GAPDH (glyceraldehyde-3-phosphate dehydrogenase), which is known to be expressed homeostatically. As a result thereof, PLD4 mRNA exhibited relatively high expression in the spleen and the peripheral blood leucocyte. In addition, it was revealed that PLD4 mRNA was expressed widely in other tissues. However, the expression level of the PLD4 mRNA was less than 100 folds of the expression level of the resting pDC cell (FIG. 6).

20 [0095]

Preparation of human PLD4 expression vector

Preparation of PLD4 gene expression vector was performed in order to express a human PLD4 protein. From PLD4 cDNA clone incorporated into a pCR4-TOPO cloning vector (Open Biosystem, Cat, No. MHS4771-99610856), only PLD4 gene was taken out with EcoRI enzyme, and incorporated into a pcDNA3.1 expression vector (human PLD4-pcDNA3.1 vector). Using the obtained human PLD4-pcDNA3.1 plasmid as a template, the PLD4 gene was amplified with a primer containing EcoRI, Not I, and Kozak sequences (GCC GCC ACC) (The information of the primers is as described below). The PCR product was cloned at EcoRI and Not I sites with pMX-IP retrovirus vector (human PLD4-pMX-IP retrovirus vector). In the PCR reaction, 1 unit of KOD Plus DNA polymerase (TOYOBO company) was used, and the reaction conditions were 1 cycle at 94°C for 2 minutes, and then 25 cycle of [94°C for 15 seconds and 68°C for 1 minutes 30 seconds].

Forward primer (SEQ ID NO: 53): 5' ttt GAA TTC gcc gcc acc ATG CTG AAG CCT 3' (30-mer)

Reverse primer (SEQ ID NO: 54): 5' aaa gcg gcc gcT CAG CCC TGC CAA ACG
CAG TCC T 3' (34-mer)

Along with the sequence analysis, HEK (Human Embryonic Kidney)-293T cell
(Hereinafter, 293T cell) was transiently transfected with human PLD4-pMX-IP retrovirus vector,
5 and whether the human PLD4 was expressed on the surface of the 293T cell was confirmed with
cell staining, and then FACS method.

Example 2

Review of specificity of anti-human PLD4 antibody

The fact that the anti-PLD4 monoclonal antibody does not cross with other
10 molecules of PLD family, can be confirmed employing a cell in which each of the PLD family is
mandatorily expressed. Human PLD4 belongs to PLD family, and there are multiple molecules
having high homology. Human PLD4 illustrates about 41% homology to human PLD3 and
about 29.3% homology to human PLD5 (FIG. 4).

1) Preparation of expression vectors of human PLD3 and human PLD5

15 For human PLD3 (cDNA SEQ ID NO: 55, amino acid SEQ ID NO: 127) and
human PLD5 (cDNA SEQ ID NO: 56, amino acid SEQ ID NO: 123), from human PLD3 clone
(K.K.DNAFORM company, Cat. No 5189261) incorporated into pCMV-SPORT6 vector, and
human PLD5 clone (K.K.DNAFORM company, Cat. No 40025860) incorporated into pCR-
Blunt II-TOPO vector, only PLD3 and PLD5 genes were amplified with PCR, and each of the
20 genes was cloned at Hind III and EcoRI sites of Flag-tagged pcDNA3.1 (Invitrogen company),
whereby to prepare an expression vector (The information of the primers is as described below).

(For human PLD3)

Forward primer: huPLD3-IF (Hind III)

25 Sequence: 5' ttt AAG CTT gcc gcc acc ATG AAG CCT AAA CTG ATG TAC 3'
(39-mer) SEQ ID NO: 57)

Reverse primer: huPLD4-1518R (EcoRI)

Sequence: 5' ttt gaa ttc TCA ctt atc gtc gtc atc ctt gta atc GAG CAG GCG GCA
GGC GTT GCC 3' (57-mer) (SEQ ID NO: 58)

(For human PLD5)

30 Forward primer: huPLD5-IF (Hind III).

Sequence: 5' ttt AAG CTT gcc gcc acc ATG GGA GAG GAT GAG GAT GGA 3'
(39-mer) (SEQ ID NO: 59)

Reverse primer: huPLD5-1383R (EcoRI)

Sequence: 5' ttt gaa ttc TCA ctt atc gtc gtc atc ctt gta atc TAC GTT CCG GGG
ATC CTT TCC 3' (57-mer) (SEQ ID NO: 60)

Among the aforementioned primer sequences, the underlined parts represent base sequences that encode the added FLAG tags, and the italic type represents Hind III cutting site or
5 EcoRI site of the restriction enzymes.

Along with the sequence analysis, 293T cell was transiently transfected with human PLD3-pcDNA3.1 vector or human PLD5-pcDNA3.1 vector, and whether the human PLD4 was expressed on the surface of the 293T cell was confirmed with cell staining, and then FACS method.

10 As a result thereof, the antibodies did not react with a cell in which human PLB3 or human PLD5 appeared to be expressed, which suggested that these anti-PLD4 antibodies recognized human PLD4 molecules specifically (FIG. 8).

[0096]

Preparation of human PLD4 expression cell stable strain

15 The prepared human PLD4-pMX-IP vector was cotransfected to 293T cell along with pCL-Eco vector (IMGEX, Cat. No. 10045P), which is a retrovirus packaging vector, whereby to perform gene introduction. The gene introduction experiment used FuGENE (registered trademark) HD Transfection Reagent (Roche) as the transfection reagent. After 2 days, the cell culture supernatant, in which human PLD4 gene-containing retrovirus was
20 secreted, was collected, and was infected to CT125 cell strain (2B4 mouse T cell lymphocyte tumor cell series). From the fact that pMX-IP retrovirus vector contains puromycin-resistant gene, culture of infected CT125 cell in the presence of puromycin allows survival of only cells that express human PLD4, leading to selection thereof. The selected human PLD4 expression CT125 cells (hereinafter, human PLD4-CT125) were further selected to only the CT125 cells
25 allowing higher expression of human PLD4 by FACS sorting, and cultured. For confirmation of the human PLD4 expression, the CT125 cells were stained with commercially available mouse anti-human PLD4 polyclonal antibody (Abnova, Cat #:HOO122618-B01P) adjusted to 5 µg/mL, and FACS analysis was performed. As a result thereof, human PLD4-CT125 cell stable strain was established, and used in FACS screening of the hybridoma.

30 [0097]

Construction of PLD4 expression vectors of cynomolgus monkey and rhesus monkey, and preparation of human PLD4 expression cell stable strain

For expressions of the PLD4 proteins of cynomolgus monkey and rhesus monkey,

cloning of monkey PLD4 gene and construction of expression vectors were performed.

1) Cloning of cynomolgus monkey PLD4 and rhesus monkey PLD4 gene

The cDNA sequence of PLD4 of rhesus monkey is reported at Genbank database (XM_002805227.1) and the like, but a partial,

5 total length cDNA thereof is not reported. Furthermore, since the PLD4 gene sequence of cynomolgus monkey is not yet reported, from PBMC (10 mL respectively; SHIN NIPPON BIOMEDICAL LABORATORIES, LTD.) of cynomolgus monkey and rhesus monkey, gene cloning was performed.

10 Total RNA was extracted from the peripheral blood of the monkey, and from 5 µg thereof, cDNA was synthesized using oligo-dT primer and SuperScript Choice System for cDNA Synthesis kit.

Using the prepared cDNA as a template, the cynomolgus monkey PLD4 and the rhesus monkey PLD4 gene were amplified with PCR method using the primers of the following base sequences.

15 Forward primer (cynoPLD4-32F): 5' AGA TGC TGA AGC CTC TTC GGA GAG Cg 3' (SEQ ID NO: 61)

Reverse primer (cynoPLD4-1554R): 5' TCA GCC CTG CCA AAC GCA GTC CTG G3' (SEQ ID NO: 62)

20 Amplified about 1521 base pairs of cynomolgus monkey PLD4 and 1521 base pairs of rhesus monkey PLD4 cDNA fragment were isolated by electrophoresis using 1% agarose gel, and collected, and cloned to pCR4Blunt-TOPO plasmid vector (Invitrogen company) using Zero Blunt TOPO PCR Cloning kit (Invitrogen company). The base sequences of the obtained gene were analyzed, which are represented by SEQ ID NO: 63 and SEQ ID NO: 124. It was confirmed that intended cynomolgus monkey PLD4 and rhesus monkey PLD4 gene were able to
25 be cloned.

The human PLD4 protein represents about 94.4% identity to the protein sequence with cynomolgus monkey PLD4 (SEQ ID NO: 129), and about 94% identity to the protein sequence with rhesus monkey PLD4 (SEQ ID NO: 130). FIG. 14 illustrates the homology of the protein sequences of human PLD4 to cynomolgus monkey PLD4, rhesus monkey PLD4, and
30 mouse PLD4 (cDNA SEQ ID NO: 131, amino acid SEQ ID NO: 132).

2) Preparation of cynomolgus monkey PLD4 expression cell stable strain

Cynomolgus monkey PLD4 expression cell stable strain was established by culturing CT125 cell infected with a retrovirus vector in the presence of puromycin, in the same method as the method of preparing human PLD4 expression cell stable strain using the prepared

cynomolgus monkey PLD4-pMX-IP vector.

For confirmation of PLD4 expression of cynomolgus monkey, the cells were stained with commercially available mouse anti-human PLD4 polyclonal antibody (Abnova, Cat #: H00122618-B01P) that represent cross-reactivity also with monkey PLD4, and FACS analysis was performed. As a result thereof, cynomolgus monkey PLD4-CT125 cell stable strain was established, and used in FACS screening of the hybridoma (FIG. 16).

[0098]

Preparation of human PLD4-Ig fusion protein

For use as an immunogen in preparation of anti-human PLD4 monoclonal antibody, 2142 bps of a DNA fragment, in which the extracellular region of human PLD4 protein (56-506 a.a) and mouse IgG2a Fc fragment (234 a.a containing a portion of the heavy chain hinge, CH2, and CH3) were fused, were amplified with 2 step PCR method, and expression vector plasmids of human PLD4-Ig pcDNA3.1 and human PLD4-Ig pEE14.4 were constructed (The sequences of the cDNA and the protein are specified in the sequence list).

In order to obtain a protein from the culture supernatant, Maxi-prep DNA was transiently transfected to Freestyle 293F cell (hereinafter, 293F cell, catalog No. R790-07; Invitrogen). At day 7 after the transfection, the culture liquid of the cotransfected 293F cell was collected in 50 mL tube, and centrifuge was performed under conditions of 2,070 g at 4°C for 5 minutes. The supernatant was filtered with a syringe filter having 0.45 µm pore size (catalog No. 431220; CORNING), and the culture supernatants were collected together.

The collected cell culture supernatant was purified with protein A affinity column of AKTA-FPLC system, and the protein of recombinant human PLD4-Ig fusion protein was purified (FIG. 7).

[0099]

Example 3

A. Preparation of anti-human PLD4 monoclonal antibody

A-1) Immunization

As an immunogen, the aforementioned recombinant PLD4-Ig fusion protein was used. PLD4-Ig fusion protein was administered subcutaneously to the back part of 3 mice of BALB/c mouse. Freund's Adjuvant, Complete and Incomplete (SIGMA) were used as an adjuvant, and 200 µg/mouse at the first time and 50 µg/mouse at the second to fourth time, were administered.

A-2) Confirmation of anti-serum titer

The blood was collected after the third immunization and the fourth

immunization, and the anti-PLD4-Ig titer in the serum was evaluated with ELISA.

PLD4-Ig fusion protein was solid-phased to a 96 well microtiter plate. The anti-serum was diluted stepwise from 1,000 folds by 3 folds, and dilution series were prepared to 729,000 folds. Each of the samples was added to the antigen solid-phased plate by 50 μ L, and primary reaction was performed. After washing, secondary reaction was performed with HRP label anti-mouse IgG (κ , λ) antibody, and color-detected (490 nm) with OPD (ortho-phenylenediamine).

[0100]

A-3) Cell fusion

Splenic cells were isolated from a mouse for which increase of the anti-serum titer was recognized. The isolated splenic cell and the mouse myeloma cell (P3U1) were fused with PEG method, and selection and culture of the fusion splenic cell was performed from HAT medium.

[0101]

FACS screening of hybridoma using CAL-1 cell

Antibodies producing each clone of the fusion splenic cell obtained from HAT selection culture were evaluated with FACS. Human pDC-like cell strain CAL-1 cells in 2×10^5 were reacted with 50 μ L culture supernatant of each of the hybridomas described below for 15 minutes at 4°C. The cells were washed with FACS buffer (1% FBS + PBS) twice, centrifuged, and the supernatant was removed. Reaction was performed for 20 minutes at 4°C using PE labeled anti-mouse IgG antibody (BD Bioscience: 550589) as a secondary antibody. The culture liquid after 10 days from the start of HAT selection culture was used as original fold for the culture supernatant of each clone. As a result thereof, 3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, 14C1, and 11G9.6 of the hybridoma culture supernatants were well reacted to CAL-1 cell (FIG. 11).

[0102]

FACS screening of hybridoma using human PLD4-CT125 stable cell strain

Antibodies producing each clone of the fusion splenic cell obtained from HAT selection culture were evaluated with FACS. Human PLD4-CT125 in 2×10^5 was reacted for 15 minutes at 4°C with 50 μ L of culture supernatant of each of the hybridomas described below. The cells were washed with FACS buffer (1% FBS + PBS) twice, centrifuged, and the supernatant was removed. Reaction was performed for 20 minutes at 4°C using PE labeled anti-mouse IgG antibody (BD Bioscience: 550589) as a secondary antibody. As a result

thereof, 3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, 14C1, and 11G9.6 of the hybridoma culture supernatants were well reacted to human PLD4-CT125 cell (FIG. 12).

[0103]

A-5) FACS screening using human peripheral blood pDC

5 [Isolation of human PBMC]

20 mL of peripheral blood of a healthy individual was collected, and the peripheral blood mononuclear cell (PBMC) was isolated with specific gravity centrifuge using HISTOPAQUE-1077 (SIGMA company). 1×10^6 PBMCs were stained for every sample. The cells were washed with FACS buffer, and then added with 25 μ L of 5 fold dilution of Fc block reagent (Miltenyi company), and reacted at 4°C for 15 minutes. The cells were washed with FACS buffer, and then was added with 50 μ L of the cell culture supernatant of each hybridoma and mouse IgG2b, κ , and reacted at 4°C for 20 minutes. The cells were washed with FACS buffer, and then added with PE-labeled anti-mouse IgG antibody, and reacted at 4°C for 20 minutes. The cells were washed with FACS buffer, and then added with 50 μ L of 10 fold dilution of APC-labeled anti-BDCA2 antibody, and reacted with at 4°C for 20 minutes. The cells were washed with FACS buffer, and then resuspended in 300 μ L of FACS buffer, and analyzed with FACS Calibur (BD). The mp11G9.6 antibody exhibited the binding to pDC (FIG. 10).

Furthermore, 9 kinds of PLD4 antibodies of 3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, and 14C1 exhibited specific binding reaction for pDC cell population, which was a BDCA2 positive cell (FIG. 13).

[0104]

A-6) Cross-reactivity of anti-PLD4 antibody to monkey

25 FACS screening of hybridoma using cynomolgus monkey PLD4-CT125 stable cell strain and rhesus monkey PLD4-293T transient transfectant cell

Antibodies producing each clone of the fusion splenic cell obtained from HAT selection culture were evaluated with FACS. Human PLD4-CT125 in 2×10^5 was reacted with 50 μ L culture supernatant of each of the hybridomas described below for 15 minutes at 4°C. The cells were washed with FACS buffer (1% FBS + PBS) twice, centrifuged, and the supernatant was removed. Reaction was performed for 20 minutes at 4°C using PE labeled anti-mouse IgG antibody (BD Bioscience: 550589) as a secondary antibody. As a result thereof, 7 kinds antibodies of 3B4, 5B7, 7B4, 13D4, 13H11, 14C1, and 11G9.6 antibodies among 10 kinds of the PLD4 antibodies were well reacted to cynomolgus monkey PLD4-CT125

cell and rhesus monkey PLD4-293T cell (FIG. 15, FIG. 16, and FIG. 17).

A-7) Cross-reactivity of anti-PLD4 antibody to monkey PBMC

PBMC of rhesus monkey from the peripheral blood (10 mL; SHIN NIPPON BIOMEDICAL LABORATORIES, LTD.), was centrifuged with specific gravity using 96% Ficoll-Paque (trademark) PLUS (GE Healthcare company, Cat No. 17-1440-02). For FACS, 5×10⁵ of cells were used per sample. The cells were washed with FACS buffer cell, and then added with 10 μL of 10% cynomolgus monkey serum diluted with FACS buffer, and reacted at 4°C for 20 minutes. The cells were washed with FACS buffer, and then added with 100 μL of the cell culture supernatant of each hybridoma and 10 μg/mL of mouse IgG2a, κ or mouse IgG1, κ, and reacted at 4°C for 15 minutes. The cells were washed with FACS buffer, and then added with 1 μg/mL of APC-labeled anti-mouse IgG antibody, and reacted at 4°C for 20 minutes. The cells were washed with FACS buffer, and then reacted with 25 μL of 10 fold dilution of FITC-labeled anti-Lineage 1 antibody, PE-labeled anti-CD123 antibody, and PcrCP-Cy5.5-labeled anti-HLA-DR antibody at 4°C for 15 minutes. The cells were washed with FACS buffer, and then resuspended in 300 μL of FACS buffer, and analyzed with FACS calibur. The used hybridoma culture supernatant was specific to PLD4, and 10 kinds of 3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, 14C1, and 11G9.6, which bound well to CAL-1 cell or human pDC, were selected. As a result thereof, 5 kinds of the hybridoma cell culture supernatants, i.e., 5B7, 7B4, 13D4, 13H11, and 14C1 specifically bound to pDC cell population of cynomolgus monkey (Lineage-CD123+ HLA-DR+) (FIG. 18).

[0105]

A-8) Cloning of hybridoma by limiting dilution method

1) Cloning and 2nd screening by limiting dilution method

For cloning of selected 9 kinds (3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, and 14C1) of hybridomas except 11G9.6 hybridoma, limiting dilution was performed. The limiting dilution was seeded on 2 pieces of 96 well plate. After 6 days from the seeding, the cells were observed under a microscope, and culture supernatants that were monoclonal-derived and exhibited good growth in all wells were collected. FACS analysis was performed for the collected culture supernatant as a sample.

In FACS analysis, the surface antigen of cell strain CAL-1 was stained using the culture supernatant of each clone followed by PE-labeled anti-mouse IgG antibody (BD Bioscience: 550589) as a secondary antibody. As the culture supernatant of each clone, the

culture liquid after 7 days from the seeding of the limiting dilution was used as original fold.

2) Cloning and 3rd screening

Based on FACS analysis results of the 2nd screening and the cell state in each well, 1 well was selected from each clone, and limiting dilution was performed again. The limiting dilution was similarly performed as in 2), and FACS analysis (3rd) was performed for the collected culture supernatant as a sample. Based on the FACS analysis data and the like, following 9 kinds (3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, and 14C1) of the clones were cloned by limiting dilution method, and anti-human PLD4 antibody-producing hybridoma was established as a stable cell strain.

10 [0106]

3) Conversion to single 11G9.6 hybridoma

The 11G9.6 hybridoma other than the aforementioned 9 kinds was collected, and suspended with a sorting buffer (1% FBS/PBS) to be 1×10^5 cells/mL. Single cell sorting was performed using FACS Aria (BD). The data were incorporated, and the incorporated data was developed to two dimensional dot plot of X axis: FSC and Y axis: SSC. On the dot plot, live cells were surrounded with a gate. The gate was draped to exclude doublet from the cells in the live cell gate, the cell population was isolated and taken into 96 well flat plate to be 1 cell/well. The cells after Single cell sorting were cultured in HAT medium (RPMI 1640+ 2 mM L-Glutamine, 10 Unit/mL Penicillin-Streptomycin, 10 mM HEPES, 1 mM Sodium Pyruvate, 50 μ M 2-ME) + hybridoma growth supplement HFCS (Roche company). Then, CAL-1 cell, human PLD4-CT125 expression stable cell strain, human PBMC, and the monkey PLD4-CT125 expression stable cell strain were stained using the cell culture supernatant of the hybridoma, and 11G9.6 of single hybridoma was selected.

[0107]

25 4) Preparation of frozen cell vial and collection of culture supernatant

Based on the FACS analysis results described above and the cell state of each well, 1 well was selected from each clone. For the selected well, expansion culture was performed at 50 mL scale. The medium was RPMI 1640 containing 10% FCS and penicillin streptomycin. The cells were cultured to subconfluent, and freezed and conserved in cell number 1×10^6 cells/tube. As the liquid for freezing and conserving, BAMBANKER (NIPPON Genetics, Co. Ltd.) was used. In addition, the culture supernatant at this time was collected and conserved.

[0108]

Example 4

Purification of antibody

10 Kinds (3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, 14C1, and 11G9.6) of purified antibodies were obtained from the culture supernatants of the hybridomas by purification using protein A affinity column (rProtein A Sepharose Fast Flow (catalog No. 17-1279-01, GE Healthcare company). Isotypes were confirmed using Pierce Rapid ELISA Mouse mAb Isotyping Kit (Thermo Fisher Scientific company). As a result thereof, 3B4 and 14C1 were mouse IgG1, κ , 10C3 was mouse IgG2a, κ , and the others were mouse IgG2b, κ .

Measurement of endotoxin concentration was performed since if endotoxin was contained in the purified antibody, it could have influence on the results of a property determination test. The Kits used were Endospecy ES-50M set, Toxicolor DIA-MP set, and endotoxin standard CSE-L set (SEIKAGAKU BIOBUSINESS CORPORATION company). As a result thereof, endotoxin concentration of any purified antibody was 0.3 EU/mg or less Ab that was the reference value.

[0109]

Review of reactivity of purified antibody

15 Binding ability of the purified antibody was confirmed with CAL-1 cell that is human pDC-like cell strain. As a result, it could be confirmed that any antibody maintained the binding ability to human PLD4 on the cell surface (FIG. 11). In addition, the antibody also specifically bound to pDC cell population (BDCA2+) of the human peripheral blood (FIG. 13).

[0110]

Calculation of Kd value of purified PLD4 antibody

20 For binding ability of the purified antibody, human PLD4-CT125 expression stable cell strain was reacted to nearly 100% of the positive staining rate at low concentration to high concentration (0.001 $\mu\text{g/mL}$ to 30 $\mu\text{g/mL}$) of purified PLD4 antibody concentration. The frequency of staining positive cell and the antibody were data-analyzed using Graph Pad Prism version 5 software, and the dissociation constant molar concentration (Kd value) was computed in nM unit. From the fact that Kd values (nM) of the anti-PLD4 antibodies were all 1 nM or less or 1 nM nearly except the 2 clones of 3B4 and 14C1, the anti-PLD4 antibodies bound very strongly to the human PLD4-CT125 cell (FIG. 19).

[0111]

30 Example 5

Complement dependent cytotoxicity activity of anti-PLD4 antibody for human PLD4-CT125 expression cell

The complement dependent cytotoxicity activity (hereinafter, referred to as CDC activity) of the anti-PLD4 antibody was measured for CT125 cell stable strain that expresses

human PLD4 (hereinafter, referred to as HuPLD4-CT-125) using immature rabbit serum as a complement source. The index of the activity was cell toxicity calculated from measured value of Lactase dehydrogenase (LDH) released from the cell. Each cell was dispensed to 96 well U bottom plate by 2×10^4 cells/50 μ L/well. 1% Baby rabbit complement (CEDARLANE company) was prepared at CDC medium (RPMI 1640 + 0.1% BSA + 10 mM HEPES + 2 mM L-Glutamine + 1% Pen-Strep). The cells were added with 10 μ g/mL of mouse isotype control antibody (mouse IgG2b, κ) and 10 kinds of the anti-PLD4 mouse purified antibodies (3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, 14C1, and 11G9.6), and reacted for 1 hour. For the assay system, CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega company) Kit was used. As a result thereof, for the target cell of HuPLD4-CT-125, 8 kinds of the PLD4 antibodies (5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, and 11G9.6) exhibited CDC activity of from about 33.5% to 71.1% at 10 μ g/mL of antibody concentration except 2 kinds of the PLD4 antibodies, 3B4 and 14C of which the heavy chain isotype was mouse IgG1 (FIG. 20).

Example 6

Concentration-dependent, complement-dependent cytotoxicity activity mouse isotype control antibody (mouse IgG2b, κ) of anti-PLD4 antibody (11G9.6), anti-PLD4 mouse antibody (mp11G9.6 Ab), human isotype control antibody (human IgG1, κ), and anti-PLD4 chimeric antibody (ch11G9 Ab) were adjusted to total 6 points of the antibody concentration of 0.1 μ g/mL to 30 μ g/mL. As the assay system, CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega company) kit was used. As a result thereof, for the target cell of HuPLD4-CT-125, mp11G9.6Ab exhibited about 70% CDC activity concentration-dependently at 3 μ g/mL of the antibody concentration. On the other hand, ch11G9Ab, which was a chimeric antibody, exhibited 10% or less of the CDC activity even at 30 μ g/mL of high concentration (FIG. 21).

[0112]

Example 7

Preparation of chimeric antibody

10 Kinds were prepared as a hybridoma that produces mouse anti-PLD4 antibody, and used.

1. Confirmation of isotype of constant region

The isotype of the constant region of the mouse anti-PLD4 antibody produced from the hybridoma that produces the anti-PLD4 antibody was confirmed. From the culture supernatants of the hybridomas of 10 kinds (3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, 14C1, and 11G9.6), the isotype was confirmed using Pierce Rapid ELISA Mouse mAb Isotyping

Kit (Thermo Fisher Scientific company). As a result thereof, 3B4 and 14C1 were mouse IgG1 and mouse Ig kappa, 10C3 was mouse IgG2a and mouse Ig kappa, and the others are mouse IgG2b and mouse Ig kappa.

[0113]

- 5 2. Cloning of cDNA that encodes variable region of mouse anti-PLD4 antibody
2-1) Isolation of total RNA

From the 11G9.6 hybridoma, total RNA was isolated using a commercially available kit, "RNeasy Mini Kit" (Qiagen company, catalog No.: 74106) according to the instruction attached to the kit. It was prepared from 5×10^6 cell number of the hybridoma cell strain, and about 79 μ g of total RNA was obtained.

[0114]

2-2) Amplification and fragmentation of cDNA that encodes mouse heavy chain variable region

5 μ g of the total RNA isolated in 2-1) was used, and cDNA that encodes the mouse heavy chain variable region was amplified by 5' RACE PCR method. In the amplification, a commercially available kit, "5' RACE System for Rapid Amplification of cDNA ENDS, Version 2.0 Kit" (Invitrogen company, catalog No.: 18374-058) was used. The details are as follows. First, single-stranded cDNA was synthesized by the reverse transcription enzyme from total RNA obtained in 2-1). At this time, the antisense primer (GSP1) used was as follows. The GSP1 primers used in amplification of cDNA were used differently depending on the isotype of each mouse heavy chain.

For example, in cloning of the heavy chain variable regions of 3B4 and 14C1 hybridomas having mouse IgG1 heavy chains, the following antisense primers are used.

GSP1 primer: mu IgG1 VH-GSP1

25 Sequence: 5'-CCA GGA GAG TGG GAG AGG CTC TTC TCA GTA TGG TGG-
3' (36-mer) (SEQ ID NO: 64)

GSP2 primer: mu IgG1 VH-GSP2

Sequence: 5'-GGC TCA GGG AAA TAG CCC TTG ACC AGG CAT CC-3' (32-mer) (SEQ ID NO: 65)

30 In cloning of the heavy chain variable regions of 10C3 hybridoma having mouse IgG2a heavy chain, the following antisense primers are used.

GSP1 primer: mu IgGH γ 1-GSP1

Sequence: 5' TCC AGA GTT CCA GGT CAC TGT CAC 3' (24-mer) (SEQ ID

NO: 66)

GSP2 primer: mu IgGH γ 1-GSP2

Sequence: 5' AGG GGC CAG TGG ATA GAC AGA TGG 3' (24-mer) (SEQ ID

NO: 67)

5 In cloning of the heavy chain variable regions of 5B7, 7B4, 8C11, 11D10, 13D4, 13H11, and 11G9.6 hybridomas having mouse IgG2b heavy chain, the following antisense primers are used.

GSP1 primer: mu IgGH γ 2B-GSP1

Sequence: 5' TCC AGA GTT CCA AGT CAC AGT CAC 3' (24-mer) (SEQ ID

10 NO: 68)

GSP2 primer: mu IgGH γ 2B-GSP2

Sequence: 5' AGG GGC CAG TGG ATA GAC TGA TGG 3' (24-mer) (SEQ ID

NO: 69)

15 Furthermore, to the 3'-terminal of the single-stranded cDNA, dC, which is a nucleotide homopolymer, was added using terminal deoxynucleotidyl transferase (TdT). Then, cDNA was amplified by PCR method using an anchor primer having a nucleotide polymer complementary to dC (anchor sequence) (SEQ ID NO: 70) at the 3'-terminal, and an antisense primer (GSP2). Furthermore, cDNA was amplified by Nested PCR method using the obtained PCR product as a template and using AUAP primer (SEQ ID NO: 71) and the antisense primer
20 shown in Table 1 (GSP2). Furthermore, this PCR product was purified by 1.5% low melting point agarose method.

[0115]

Anchor primer for 5' RACE (SEQ ID NO: 70)

5'-GGC CAC GCG TCG ACT AGT ACG GGI IGG GII GGG IIG-3' 36-mer)

25

AUAP primer for 5' RACE (SEQ ID NO: 71)

5'-GGC CAC GCG TCG ACT AGT AC-3' 20-mer)

[0116]

2-3) Amplification and fragmentation of cDNA that encodes mouse light chain variable region

30 cDNA that encodes the mouse light chain variable region was amplified from the total RNA in isolated 2-1), similarly to 2-2). At this time, the GSP1 primers used in amplification of cDNA were used differently depending on the isotype of each mouse light

chain.

[0117]

The following antisense primers are used for light chain cloning since the 10 kinds of the PLD4 antibodies have mouse Ig kappa light chain.

5 GSP1 primer: mu IgG VL kappa-GSP1

Sequence: 5'-CAC TAC TTC CTG TTG AAG CTC TTG ACG ATG G-3' (31-mer) (SEQ ID NO: 72)

GSP2 primer: mu IgG VL kappa-GSP2

10 Sequence: 5'-GTG AGT GGC CTC ACA GGT ATA GC-3' (23-mer) (SEQ ID NO: 73)

The obtained PCR product was purified by 1.5% low melting point agarose method.

[0118]

2-4) Confirmation of the cDNA base sequence and Determination of CDR region

15 The cDNA fragments of the heavy chain variable region obtained in 2-2), and the light chain variable region obtained in 2-3) were cloned, respectively with pCR4Blunt-TOPO vector using a commercially available kit "Zero Blunt TOPO PCR Cloning Kit" (Invitrogen company, catalog No.: 1325137), according to the instruction attached to the kit, and transformed to Escherichia coli competent cell to obtain an Escherichia coli transformant. A plasmid was
20 obtained from this transformant, and the plasmid DNA sample was sent for sequence analysis to Operon Biotechnology Co. Ltd company (Tokyo), and the cDNA base sequence in the plasmid was confirmed. In analysis of the sequence, softwares of "Sequencher DNA sequence assembly and analysis software version 4.2.2 (Gene Codes Corporation)" and "GENETYX-MAC Version. 11.1.1" software (GENETYX CORPORATION)" were used.

25 [0119]

A transcript of a right sequence was extracted excluding a transcript of inactive RNA, as frame shift, nonsense mutation, and the like occur at the periphery of a complementarity-determining region (hereinafter referred to as "CDR region"). Furthermore, for the cDNA base sequence contained in the plasmid, the homology with Immunoglobulins
30 database (IgBLAST, URL: www.ncbi.nlm.nih.gov/Igblast/) was confirmed, and the sequences of the CDR region in each variable region (CDRs; CDR1, CDR2, and CDR3), the FW region (Frame work regions), and the variable regions were determined according to the analysis method of Kabat numbering system (Kabat et al, 1991, Sequences of Proteins of Immunological

Interest, National Institutes of Health Publication No. 91-3242, 5th ed., United States Department of Health and Human Services, Bethesda, MD).

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 11G9.6 antibody is SEQ ID NO: 74, and the amino acid sequence is SEQ ID NO: 75.

- 5 The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 11G9.6 antibody are SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, respectively.

- 10 The nucleic acid sequence of the heavy chain variable region of the obtained mouse 3B4 antibody is SEQ ID NO: 76, and the amino acid sequence is SEQ ID NO: 77. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 3B4 antibody are SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 5B7 antibody is SEQ ID NO: 78, and the amino acid sequence is SEQ ID NO: 79. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 5B7 antibody are SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively.

- 15 The nucleic acid sequence of the heavy chain variable region of the obtained mouse 7B4 antibody is SEQ ID NO: 80, and the amino acid sequence is SEQ ID NO: 81. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 7B4 antibody are SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively. The 7B4 antibody is an antibody having the same heavy chain and the light chain variable region CDR sequences as those of the 5B7 antibody.
- 20

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 8C11 antibody is SEQ ID NO: 82, and the amino acid sequence is SEQ ID NO: 83. The amino acid sequences of CDR1, CDR2 and CDR3 in the heavy chain variable region of the mouse 8C11 antibody are SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, respectively.

- 25 The nucleic acid sequence of the heavy chain variable region of the obtained mouse 10C3 antibody is SEQ ID NO: 84, and the amino acid sequence is SEQ ID NO: 85. The amino acid sequences of CDR1, CDR2 and CDR3 in the heavy chain variable region of the mouse 10C3 antibody are SEQ ID NO: 26, SEQ ID NO: 27, and SEQ ID NO: 28, respectively.

- 30 The nucleic acid sequence of the heavy chain variable region of the obtained mouse 11D10 antibody is SEQ ID NO: 86, and the amino acid sequence is SEQ ID NO: 87. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 11D10 antibody are SEQ ID NO: 26, SEQ ID NO: 27, and SEQ ID NO: 28, respectively. The 11D10 antibody is an antibody having the same light chain variable region CDR sequence to the heavy chain of the 10C3 antibody. However, the heavy chain isotype (10C3 is the constant

region of the mouse IgG2a, and 11D10 is the constant region of the mouse IgG2b) is different.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 13D4 antibody is SEQ ID NO: 88, and the amino acid sequence is SEQ ID NO: 89. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 13D4 antibody are SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 13H11 antibody is SEQ ID NO: 90, and the amino acid sequence is SEQ ID NO: 91. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 13H11 antibody are SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 14C1 antibody is SEQ ID NO: 92, and the amino acid sequence is SEQ ID NO: 93. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 14C1 antibody are SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40, respectively. The 14C1 antibody is an antibody having the same CDR sequences of the heavy chain and light chain variable regions as those of the 13H11 antibody. However, the heavy chain isotype (13H11 is the constant region of Mouse IgG2b, and 14C1 is the constant region of mouse IgG1) is different.

[0120]

The nucleic acid sequence of the light chain variable region of the mouse 11G9.6 antibody is SEQ ID NO: 94, and the amino acid sequence is SEQ ID NO: 95. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 11G9.6 antibody are SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 3B4 antibody is SEQ ID NO: 96, and the amino acid sequence is SEQ ID NO: 97. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 3B4 antibody are SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 5B7 antibody is SEQ ID NO: 98, and the amino acid sequence is SEQ ID NO: 99. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 5B7 antibody are SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 7B4 antibody is SEQ ID NO: 100, and the amino acid sequence is SEQ ID NO: 101. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 7B4 antibody are SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 8C11 antibody is SEQ ID NO: 102, and the amino acid sequence is SEQ ID NO: 103. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 8C11 antibody are SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25, respectively.

5 The nucleic acid sequence of the light chain variable region of the mouse 10C3 antibody is SEQ ID NO: 104, and the amino acid sequence is SEQ ID NO: 105. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 10C3 antibody are SEQ ID NO: 29, SEQ ID NO: 30, and SEQ ID NO: 31, respectively.

10 The nucleic acid sequence of the light chain variable region of the mouse 11D10 antibody is SEQ ID NO: 106, and the amino acid sequence is SEQ ID NO: 107. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 11D10 antibody are SEQ ID NO: 29, SEQ ID NO: 30, and SEQ ID NO: 31, respectively.

15 The nucleic acid sequence of the light chain variable region of the mouse 13D4 antibody is SEQ ID NO: 108, and the amino acid sequence is SEQ ID NO: 109. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 13D4 antibody are SEQ ID NO: 35, SEQ ID NO: 36, and SEQ ID NO: 37, respectively.

20 The nucleic acid sequence of the light chain variable region of the mouse 13H11 antibody is SEQ ID NO: 100, and the amino acid sequence is SEQ ID NO: 111. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 13H11 antibody are SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 14C1 antibody is SEQ ID NO: 112, and the amino acid sequence is SEQ ID NO: 113. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 14C1 antibody are SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, respectively.

25 [0121]

3. Preparation of expression vector of chimeric antibody 11G9.6

3-1. Cloning of cDNA that encodes human Ig constant region

From total RNA of human PBMC, cDNAs of the human IgG1 heavy chain constant region and the human Ig kappa light chain constant region were cloned, and cloned with
30 pCR4Blunt-TOPO vector, respectively using a commercially available kit "Zero Blunt TOPO PCR Cloning Kit" (manufactured by Invitrogen company, catalog No.: 1325137) according to the instruction attached to the kit, and transformed to Escherichia coli competent cell to obtain an Escherichia coli transformant. A plasmid was obtained from this transformant, and the plasmid DNA sample was sent for sequence analysis to Operon Biotechnology Co. Ltd (Tokyo), and the

cDNA base sequence in the plasmid was confirmed.

[0122]

3-2. Preparation of cDNA that encodes heavy chain of chimeric PLD4 antibody

cDNA that encodes the heavy chain of a chimeric PLD4 antibody was prepared
5 by ligation with pEE6.4 vector expression vector that has the heavy chain variable region of the
mouse 11G9.6 antibody obtained in 2-2, and the heavy chain constant region of the human IgG1.
The heavy chain variable region of the mouse 11G9.6 antibody was amplified with PCR method,
and a PCR product of about 450 base lengths was obtained. At this time, the primer is as
shown in Table 1. The obtained PCR product was purified by 1.5% low melting point agarose
10 method.

[Table 1]

Table 1

Primer name		Sequence
Chimera 11G9.6 antibody heavy chain expression primer		
1)	chi11G9VH-IF(Hind3)	5' acc AAG CTT gcc gcc acc ATG AAA GTG TTG AGT CTG TTG TAC CTG TTG ACA GCC ATT CCT GGT ATC CTG TCT cag GTC CAA CTG CAG CAG CCT 3' (93-mer) (SEQ ID NO:114)
2)	chi11G9VH-444R(ApaI)	5' cga tgg gcc ctt ggt gct agc TGA GGA GAC GGT GAC TGA GGT 3' (42-mer) (SEQ ID NO:115)
Chimera 11G9.6 antibody light chain expression primer		
5)	chi11G9VL-IF(Hind)	5' acc AAG CTT gcc gcc acc ATG ATG TCC TCT GCT CAG TTC 3' (39-mer) (SEQ ID NO:116)
6)	chi11G9VL-408R	5' agc cac agt tcg TTT GAT TTC CAG CTT GGT GCC 3' (33-mer) (SEQ ID NO:117)
7)	chi11G9VL-385F	5' CTG GAA ATC AAA cga act gtg gct gca cca tct 3' (33-mer) (SEQ ID NO:118)
8)	chi11G9VL-726R(RI)	5' aaa GAA TTC cta gca ctc tcc cct gtt gaa 3' (30-mer) (SEQ ID NO:119)

[0123]

The heavy chain variable region of the mouse 11G9.6 antibody obtained in 2-2 was subjected to PCR method to obtain "PCR Product that encodes 11G9.6 heavy chain variable region". The PCR Product that encodes the heavy chain variable region of the 11G9.6 was digested with Hind III and Apa I restriction enzymes, and purified with 1.5% agarose gel method. This was dissolved in ddH₂O, which was taken as a solution of a cDNA fragment that encodes the heavy chain variable region.

The obtained cDNA was amplified with PCR from pCR4Blunt-TOPO plasmid clone containing 11G9.6 V_H region, using primers chi11G9VH-IF (Hind III) and chi11G9VL-408R, in which the restriction sites (Hind III and Apa I) preferred for cloning of the V_H code

region of chimeric 11G9.6 to pEE6.4 vector (manufactured by Lonza Biologics, Slough, UK), and ideal Kozak sequence (GCCGCCACC) were introduced as the cloning sites of Hind III and Apa I. Chi11G9VH-pEE6.4 vector contains the heavy chain constant region of human IgG1. The V_H PCR fragment was inserted into pEE6.4 vector with an in-frame using Hind III and Apa I. The construct was investigated by cDNA base sequence analysis. For the sequence analysis, the plasmid DNA sample was sent to Operon Biotechnology Co. Ltd (Tokyo), and the cDNA base sequence in the plasmid was confirmed.

[0124]

3.3 Preparation of cDNA that encodes the light chain of chimeric PLD4 antibody

In order to prepare cDNA that encodes the light chain of the chimeric PLD4 antibody, the light chain variable region of the mouse 11G9.6 antibody obtained in 2-3, and the light chain constant region of the human Ig kappa obtained in 3-2 were fused to give a PCR fragment, and the PCR fragment was amplified to a PCR product of about 730 base lengths by an approach based on an overlap extension PCR method.

The PCR product that encodes the light chain variable region of the 11G9.6 was digested with Hind III and EcoR I restriction enzymes, and purified with 1.5% agarose gel method. This was dissolved in ddH₂O, which was taken as a solution of a cDNA fragment that encodes the light chain variable region.

[0125]

The obtained cDNA that encodes V_L of the chimeric 11G9 was amplified with PCR from pCR4Blunt-TOPO plasmid clone containing 11G9.6 V_L region, using primers chi11G9VL-IF (Hind) and chi11G9VL-726R (R I), in which the restriction sites (Hind III and EcoR I) preferred for cloning of pEE14.4 vector (manufactured by Lonza Biologics), and ideal Kozak sequence were introduced. The Chi11G9VL-pEE14.4 vector contains the light chain constant region of kappa. The V_L PCR fragment was inserted into pEE14.4 vector with an in-frame using Hind III and EcoR I. The construct was investigated by cDNA base sequence analysis.

[0126]

3.4 Construction of double gene Lonza expression vector of chimeric PLD4 antibody (chi11G9VH/VL)

The chimeric PLD4 antibody (chi11G9DG vector) Lonza expression vector combined in one 2-gene vector was constructed from chimeric PLD4 antibody heavy chain expression vector (chi11G9VH-pEE6.4), and chimeric PLD4 antibody light chain vector (chi11G9VL-pEE14.4) by standard cloning technology.

[0127]

4. Transient expression in 293F cell

80 μ g of ch11G9DG Lonza vector DNA, a transient expression vector plasmid, was used.

5 293F cells were combined to 80 mL in 8×10^5 cells/mL in 250 mL Erlenmyer flask (catalog No. 431144; manufactured by CORNING) on the previous day of transfection, and cultured at the conditions of 37°C and 8% CO₂ concentration for 7 days with shaking.

After the culture for 7 days, the culture liquid of the transfected 293F cell was collected to 50 mL tube, and centrifuge was performed at the conditions of 2,070 g at 4°C for 5
10 minutes. The supernatant was filtered with a syringe filter having 0.45 μ m pore size (catalog No. 431220; manufactured by CORNING), and the culture supernatant was collected for antibody purification.

[0128]

5. Purification of anti-PLD4 chimeric antibody

15 Using the collected culture supernatant, antibody purification was performed using AKTA-FPLC (manufactured by GE Healthcare Japan) and software Unicorn 5.0.

As the column for chimeric 11G9.6 antibody purification, HiTrap MabSelect SuRe 1 mL (catalog No. 11-0034-93, Lot No. 10032458; manufactured by GE Healthcare Japan) was used. The column conditions are as follows. Affinity purification was performed using a
20 binding buffer (20 mM Sodium phosphate, 0.15 M NaCl, pH 7.4) and an elution buffer (20 mM Sodium citrate, pH 3.4). In order to replace the buffer of the antibody after purification with PBS, buffer exchange was performed using Slide-A-Lyzer MINI Dialysis Unit 10kMWCO.

The concentration of the purified antibody was calculated by measuring the absorbance at 280 nm, wherein 1 mg/mL was calculated as 1.38 OD.

25 For the ch11G9.6Ab of the purified anti-PLD4 chimeric antibody, the protein quality was analyzed with Flow cytometry method and SDS-PAGE.

[0129]

Example 8

Antibody-dependent cellular cytotoxicity (ADCC activity) of the prepared anti-
30 human PLD4 chimeric antibody (ch11G9.6 Ab) was measured. For the activity, cell toxicity calculated from measurement value of lactase dehydrogenase (LDH) released from the cell was used an index. The human peripheral blood mononuclear cell that became an effector cell was purified with specific gravity centrifuge using HISTOPAQUE-1077. As the cell to be a target, mandatory transformed cell of human PLD4 gene employing a CHO-K1 cell strain (Chinese

hamster ovary cell strain) (Hereinafter, HuPLD4-CHO) was used (2×10^4 /well). The effector and the target cell were mixed in a ratio of 10:1, 20:1, 40:1, and 80:1, and added with 10 mg/mL of ch11G9Ab or isotype control antibody (human IgG1, κ), cultured at 37°C for 4 hours, and the effect of cytotoxicity activity of the antibody was evaluated. As a result thereof, ch11G9Ab of the anti-hPLD4 chimeric antibody exhibited maximum about 50% or so of ADCC activity to the HuPLD4-CHO cells that was the target, dependently on the effector cell (FIG. 22). Such results proved that the prepared anti-PLD4 chimeric antibody damaged the cells that expressed PLD4 selectively.

[0130]

The effects of the anti-PLD4 antibody on pDC were reviewed. PBMC from the human peripheral blood was purified, mixed with 10 μ g/mL of anti-human PLD4 chimeric antibody and cultured for 24 hours. Then, the cells were stimulated for 24 hours with CpG2216, which was a ligand of Toll-like receptor 9 expressed in pDC, to induce IFN α production. After the CpG stimulation, the amount of produced IFN α was tested, and it was confirmed that IFN α production was completely inhibited by treatment of ch11G9Ab of the anti-PLD4 chimeric antibody (FIG. 23). As for this mechanism, it was found out that when the cells were collected 24 hours after the ch11G9Ab treatment, and pDC cells were confirmed with triple staining of the anti-CD123 antibody, anti-HLA—DR antibody and anti-Lineage 1 antibody, pDC cell population decreased more than treatment of isotype control antibody (human IgG1, κ) (FIG. 24). These results indicated that the anti-PLD4 chimeric antibody damaged pDC that specifically expressed PLD4, and as a result thereof, IFN α production by CpG2216 stimulation was inhibited.

In addition to ch11G9.6Ab, biological function of the chimeric anti-PLD4 Abs such as ch3B4Ab, ch5B7Ab, ch8C11Ab, ch10C3Ab, ch13D4Ab, ch13H11Ab were examined in human primary pDCs. In order to examine ADCC assay for pDCs, whole human PBMCs were cultured with ch3B4Ab, ch5B7Ab, ch8C11Ab, ch10C3Ab, ch13D4Ab, ch13H11Ab, ch11G9.6Ab, or isotype Ab for 14 h. The cells were harvested and stained BDCA2 and BDCA4 to identify pDCs by flow cytometry. The treatment with the chimeric PLD4 Abs completely depleted pDCs compared to isotype Ab-treated PBMCs (FIG. 25). IFN α production was also measured in the culture of PBMCs with the chimeric anti-PLD4 Abs. Whole human PBMCs were treated with the ch3B4Ab, ch5B7Ab, ch8C11Ab, ch10C3Ab, ch13D4Ab, ch13H11Ab, ch11G9.6Ab, or isotype Ab. 24 h later, IFN α inducible CpG2216 was added to the culture and the cells were further cultured for 24 h. The culture supernatants were harvested and measured IFN α production by

ELISA. All of the chimeric PLD4 Ab-treated PBMCs completely abolished IFN α production compared to isotype Ab-treated PBMCs (FIG. 26). These results indicated that the chimeric anti-PLD4 Ab abolished pDC function such as a large amount of IFN α production by depleting pDCs via ADCC activity.

- 5 1. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 11G9.6 antibody is SEQ ID NO: 74, and the amino acid sequence is SEQ ID NO: 75. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 11G9.6 antibody are SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, respectively.

10 [0131]

The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 11G9.6 antibody (504 bp) [Upper case: mouse 11G9.6VH variable region, lower case: mouse IgG2b heavy chain constant region] (SEQ ID NO: 74)

ATGAGATCACAGTTCTCTATACAGTTACTGAGCACACAGAACCTCACCTTGGGATGGAGCTGTATCATCCTCTTCTTGGT
AGCAACAGCTACAGGTGTCCACTCCCAGGTCCAAGTGCAGCAGCCTGGGGCTGAACTGGTGAAGCCTGGGACTTCAGTGA
AAATGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCGGGACAAGGCCTT
GAGTGGATTGGAGATATTTATCCTGGTAGTGATAGTACTAACTACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGT
AGACACATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGAG
GAGGGTGGTTGGATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCagccaaaacaacacccccatca
gtctatccactggccccctaagggc

[0132]

- 15 The amino acid sequence of the heavy chain variable region of mouse 11G9.6 antibody (168 a.a.) [Upper case: mouse 11G9.6VH variable region, lower case: mouse IgG2b heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3) (SEQ ID NO: 75).

MRSQFSIQLLSTQNLTLGWSCIILFLVATATGVHSQVQLQQPGAELVKPGTSVKMSCK
ASGYTFTSYWMHWVKQRPGQGLEWIGDIYPGSDSTNYNEKFKSKATLTVDTSSTA
YMQQLSSLTSEDSAVYYCARGGWLDAMDYWGQGTSVTVSSaktppsvyplapkg

[0133]

- 20 CDR1 of the heavy chain variable region of 11G9.6 antibody
SYWMH (SEQ ID NO: 2)
CDR2 of the heavy chain variable region of 11G9.6 antibody
DIYPGSDSTNYNEKFKS (SEQ ID NO: 3)
CDR3 of the heavy chain variable region of 11G9.6 antibody
25 GGWLDAMDY (SEQ ID NO: 4)

[0134]

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 11G9.6 antibody is SEQ ID NO: 38, and the amino acid sequence is SEQ ID NO: 39. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 11G9.6 antibody are SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42, respectively.

[0135]

The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 11G9.6 antibody (421 bp) [Upper case: mouse 11G9.6VL variable region, lower case: mouse Igk light chain constant region] (SEQ ID NO: 94)

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGATATCCAGATGACACAGAC
TACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGACATTAGCAATTATTAA
ACTGGTATCAGCAGAAACCAGATGGAAGTGTAACTCCTGATCTACTACACATCAAGATTACACTCAGGAGTCCCATCA
AGGTTCAAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTT
TTGCCAACAGGGTAATACGCTTCCGTGGACGTTCCGGTGGAGGCACCAAGCTGGAAATCAAACggggctgatgctgcaccaa
ctgtatccatcaagggcgaat

[0136]

The amino acid sequence of the light chain variable region of the mouse 11G9.6 antibody (140 a.a.) [Upper case: mouse 11G9.6VL variable region, lower case: light chain constant region of mouse Igk]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3) (SEQ ID NO: 95).

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQ
QKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQOGNTLP
WTFGGGTKLEIKradaaptvsikge

[0137]

CDR1 of the light chain variable region of 11G9.6 antibody

RASQDISNYLN (SEQ ID NO: 5)

CDR2 of the light chain variable region of 11G9.6 antibody

YTSRLHS (SEQ ID NO: 6)

CDR3 of the light chain variable region of 11G9.6 antibody

QQGNTLPW (SEQ ID NO: 7)

2. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 3B4 antibody is SEQ ID NO: 76, and the amino acid sequence is SEQ ID NO:

77. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 3B4 antibody are SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10, respectively.

The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 3B4 antibody (437 bp) [Upper case: mouse 3B4VH variable region, lower case: mouse IgG1 heavy chain constant region]

ATGGAATGTAAGTGGATACTTCCTTTTATTCTGTCGGTAATTTTCAGGGGTCTCCTCAGAGGTTTCAGCTCCAGCAGTCTGG
GACTGTGCTGTCAAGGCCTGGGGCTTCCGTGACGATGTCCTGCAAGGCTTCTGGCGACAGCTTTACCACCTACTGGATGC
ACTGGGTAAAACAGAGGCCTGGACAGGGTCTAGAATGGATTGGTGCTATCTATCCTGGAAATAGTGAACTAGCTACAAC
CAGAAGTTCAAGGGCAAGGCCAAACTGACTGCAGTCACATCCGCCAGCACTGCCTATATGGAGTTCCTAGCCTGACAAA
TGAGGACTCTGCGGTCTATTACTGTACGGGGGGTTATTCCGACTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCT
CCTCagccaaaacgacacccccatctgtctatccact

The amino acid sequence of the heavy chain variable region of the mouse 3B4 antibody (145 a.a.) [Upper case: mouse 3B4VH variable region, lower case: mouse IgG1 heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MECNWILPFILSVISGVSEVQLQQSGTVLSRPGASVTMSCKASGDSFTTYWMHWVK
QRPQGQGLEWIGAIYPGNSETSYNQKFKGKAKLTAVTSASTAYMEFTSLTNEDSAVYY
CTGGYSDFDYWGQGTTLTVSSaktppsvyp

CDR1 of the heavy chain variable region of 3B4 antibody
TYWMH

CDR2 of the heavy chain variable region of 3B4 antibody
AIYPGNSETSYNQKFKG

15 CDR3 of the heavy chain variable region of 3B4 antibody
GYSDFDY

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 3B4 antibody is SEQ ID NO: 96, and the amino acid sequence is SEQ ID NO: 97. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 3B4 antibody are SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13, respectively.

The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 3B4 antibody (459 bp) [Upper case: mouse 3B4VL variable region, lower case: mouse Igk light

chain constant region]

ATGATGGTCCTTGCTCAGTTTCTTGCAATTCTTGTTGCTTTGGTTTCCAGGTGCAGGATGTGACATCCTGATGACCCAATC
TCCATCCTCCATGTCTGTATCTCTGGGAGACACAGTCAGCATCACTTGCCATGCAAGTCAGGGCATTAGAAGTAATATAG
GGTGGTTGCAGCAGAAACCAGGGAAATCATTTAAGGGCCTGATCTTTCATGGAACCAACTTGGAAGATGGAGTTCATCA
AGGTTCAGTGGCAGAGGATCTGGAGCAGATTATTCTCTCACCATCAACAGCCTGGAATCTGAAGATTTTGCAGACTATTA
CTGTGTACAGTATGTTTCAGTTTCTCCAACGTTCCGGCTCGGGGACAAAGTTGGAAATAAGAcgggctgatgctgcaccaa
ctgtatccatcttcccaccateccagtgagcagttaacatctggaggtgcctcagtcgtg

The amino acid sequence of the light chain variable region of the mouse 3B4 antibody (153 a.a.) [Upper case: mouse 3B4VL variable region, lower case: mouse Igk light chain constant region]. The underlined sequence represents a signal sequence, and the double
5 underline represents CDR regions (CDR1, CDR2, and CDR3).

MMVLAQFLAFLLLWFPGAGCDILMTQSPSSMSVSLGDTVSITHASQGIRSNIGWLQ
QKPGKSFKGLIFHGTNLEDGVPSRFSGRGSGADYSLTINSLESEDFADYYCVQYVQFP
PTFGSGTKLEIRradaaptvsifppsseqltsggasvv

CDR1 of the light chain variable region of 3B4 antibody

HASQGIRSNIG

CDR2 of the light chain variable region of 3B4 antibody

HGTNLED

10 CDR3 of the light chain variable region of 3B4 antibody

VQYVQFP

3. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 5B7 antibody is SEQ ID NO: 78, and the amino acid sequence is SEQ ID NO: 79. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region
15 of the mouse 5B7 antibody are SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively.

The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 5B7 antibody (475 bp) [Upper case: mouse 5B7VH variable region, lower case: mouse IgG2b heavy chain constant region]

ATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCAGGCGTCCACTCTGAGGTCCAGCTTCAGCAGTCAGG
 ACCTGAACTGGTGAAACCTGGGGCCTCAGTGAAGATATCCTGCAAGGCTTCTGGATACACATTCAGTACTACAACCTGC
 ACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTGGATATATTTATCCTTACAATGGTAATACTGGCTACAAC
 CAGAAGTTCAAGAGGAAGGCCACATTGACTGTAGACAATTCCTCCGGCACAGTCTACATGGAGCTCCGCAGCCTGACATC
 TGAGGACTCTGCAGTCTATTACTGTGCAAGAGGAGGGATCTATGATGATTACTACGACTATGCTATCGACTATTGGGGTC
 AAGGAACCTCAGTCACCGTCTCCTCagccaaaacaacacccccatcagtcctatccactggccccctaaaggcgaaat

The amino acid sequence of the heavy chain variable region of the mouse 5B7 antibody (158 a.a.) [Upper case: mouse 5B7VH variable region, lower case: mouse IgG2b heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MGWSWIFLFLLSGTAGVHSEVQLQQSGPELVKPGASVKISCKASGYTFTDYNLHWV
KQSHGKSLEWIGYIYPYNGNTGYNQKFKRKATLTVDNSSGTVYMELRSLTSEDSAV
YYCARGGIYDDYYDYAIDYWGQGTSTVTVSSaktppsvyplapkgc

5 CDR1 of the heavy chain variable region of 5B7 antibody

DYNLH

CDR2 of the heavy chain variable region of 5B7 antibody

YIYPYNGNTGYNQKFKR

CDR3 of the heavy chain variable region of 5B7 antibody

10 GGIYDDYYDYAIDY

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 5B7 antibody is SEQ ID NO: 98, and the amino acid sequence is SEQ ID NO: 99. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 5B7 antibody are SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

15 The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 5B7 antibody (467 bp) [Upper case: mouse 5B7VL variable region, lower case: mouse Igk light chain constant region]

ATGAGTGTGCCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGATGCCAGATGTGACATCCAGATGACTCAGTC
 TCCAGCCTCCCTATCTGTATCTGTGGGAGAACTGTCGCCATCACATGTGCGAGCAAGTGAGAATATTTACAGTCATATAG
 CATGGTATCAGCAGAAAGAGGGGAAAATCTCCTCAGCGCCTGGTCTATGGTGCAACAACTTAGCACATGGTGTGCCATCA
 AGGTTCAGTGGCAGTGGATCAGGCACACAGTATTCCTCAAGATCAACAGCCTTCAGTCTGAAGATTTTGGGAGTTATTA
 CTGTCAACATTTTTGGGGTACTCCGTGGACGTTCCGTGGAGGCACCAAGCTGGAAATCAAacgggctgatgctgcaccaa
 ctgtatccatcttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgtgcttctt

The amino acid sequence of the light chain variable region of the mouse 5B7

antibody (155 a.a.) [Upper case: mouse 5B7VL variable region, lower case: mouse Igk light chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MSVPTQVLGLLLLWLTDARCDIQMTQSPASLSVSVGETVAITCRASENIYSHIAWYQ
QKEGKSPQRLVYGATNLAHGVPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQHFWGT
PWTFGGGTKLEIKradaaptvsifppsseqltsggasvvcf

CDR1 of the light chain variable region of 5B7 antibody

5 RASENIYSHIA

CDR2 of the light chain variable region of 5B7 antibody

GATNLAH

CDR3 of the light chain variable region of 5B7 antibody

QHFWGTP

10 4. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 7B4 antibody is SEQ ID NO: 80, and the amino acid sequence is SEQ ID NO: 81. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 7B4 antibody are SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively.

15 The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 7B4 antibody (470 bp) [Upper case: mouse 7B4VH variable region, lower case: mouse IgG2b heavy chain constant region]

ATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCAGGCGTCCACTCTGAGGTCCAGCTTCAGCAGTCAGG
 ACCTGAACTGGTGAAACCTGGGGCCTCAGTGAAGATATCCTGCAAGGCTTCTGGATACACATTCAGTGAACAACCTTGC
 ACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTGGATATATTTATCCTTACAATGGTAATACTGGCTACAAC
 CAGAAGTTCAAGAGGAAGGCCACATTGACTGTAGACAATTCCTCCGGCACAGTCTACATGGAGCTCCGCAGCCTGACATC
 TGAGGACTCTGCAGTCTATTACTGTGCAAGAGGAGGGATCTATGATGATTACTACGACTATGCTATCGACTATTGGGGTC
 AAGGAACCTCAGTCACCGTCTCCTCagccaaaacaacacccccatcagtcctatccactggccccctaaggg

20 The amino acid sequence of the heavy chain variable region of the mouse 7B4 antibody (156 a.a.) [Upper case: mouse 7B4VH variable region, lower case: mouse IgG2b heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MGWSWIFLFLLSGTAGVHSEVQLQQSGPELVKPGASVKISCKASGYTFTDYNLHWV
KQSHGKSLEWIGYIYPYNGNTGYNQKFKRKATLTVDNSSGTVYMELRSLTSEDSAV
YYCARGGGIYDDYYDYAIDYWGQGTSTVTVSSaktppsvyplapk

CDR1 of the heavy chain variable region of 7B4 antibody

DYNLH

CDR2 of the heavy chain variable region of 7B4 antibody

YIYPYNGNTGYNQKFKR

5 CDR3 of the heavy chain variable region of 7B4 antibody

GGIYDDYYDYAIDY

10 The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 7B4 antibody is SEQ ID NO: 100, and the amino acid sequence is SEQ ID NO: 101. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 7B4 antibody are SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 7B4 antibody (454 bp) [Upper case: mouse 7B4VL variable region, lower case: mouse Igk light chain constant region]

ATGAGTGTGCCCCTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGATGCCAGATGTGACATCCAGATGACTCAGTC
TCCAGCCTCCCTATCTGTATCTGTGGGAGAACTGTCGCCATCACATGTGAGCAAGTGAGAATATTACAGTCATATAG
CATGGTATCAGCAGAAAGAGGGAAAATCTCCTCAGCGCCTGGTCTATGGTGCAACAACTTAGCACATGGTGTGCCATCA
AGGTTTCAGTGGCAGTGGATCAGGCACACAGTATTCCCTCAAGATCAACAGCCTTCAGTCTGAAGATTTTGGGAGTTATTA
CTGTCAACATTTTGGGGTACTCCGTGGACGTTCCGTGGAGGCACCAAGCTGGAAATCAAACggggctgatgctgcaccaa
ctgtatccatcttcccaccatccagtgagcagttaacatctggaggtgcctcag

15 The amino acid sequence of the light chain variable region of the mouse 7B4 antibody (151 a.a.) [Upper case: mouse 7B4VL variable region, lower case: mouse Igk light chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MSVPTQVLGLLLLWLTDARCDIQMTQSPASLSVSVGETVAITCRASENIYSHIAWYQ
QKEGKSPQRLVYGATNLAHGVPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQHFWGT
PWTFGGGTKLEIKradaaptvsifppsseqltsggas

CDR1 of the light chain variable region of 7B4 antibody

RASENIYSHIA

CDR2 of the light chain variable region of 7B4 antibody

GATNLAH

CDR3 of the light chain variable region of 7B4 antibody

QHFWGTP

5

5. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 8C11 antibody is SEQ ID NO: 82, and the amino acid sequence is SEQ ID NO: 83. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 8C11 antibody are SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22, respectively.

10

The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 8C11 antibody (462 bp) [Upper case: mouse 8C11VH variable region, lower case: mouse IgG2b heavy chain constant region]

ATGGGATGGAGCTATATCATCCTCTTTTTGGTAGCAACAGCAACAGGGGTCCACTCCCAGGTCCAAGTGCAGCAGTCGGG
GGCTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGTTGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTATTTGT
ACTGGGTGAGGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGACTGATTAATCCTACCAATAGTGATACTATCTTCAAT
GAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAAATCCTCCAGCACAGCATACATGCAACTCAGCAGCCTGACATC
TGAGGACTCTGCGGTCTATTACTGTACACGAGAGGGGGGATATGGTTACGGCCCGTTTGCTTACTGGGGCCAAGGGACTC
TGGTCACTGTCTCTGCagccaaaacaacaccccccatcagtcctatccactggccccctaagggc

The amino acid sequence of the heavy chain variable region of the mouse 7B4 antibody (154 a.a.) [Upper case: mouse 8C11VH variable region, lower case: mouse IgG2b heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

15

MGWSYIILFLVATATGVHSQVQLQQSGAELVKPGASVKLSCKASGYTFTSYLYWV
RQRPGQGLEWIGLINPTNSDTIFNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYY
CTREGGYGYGPFAYWGQGTLVTVSAakttppsvyplapkg

CDR1 of the heavy chain variable region of 8C11 antibody

SYLY

CDR2 of the heavy chain variable region of 8C11 antibody

LINPTNSDTIFNEKFKS

CDR3 of the heavy chain variable region of 8C11 antibody

EGGYGYGPFAY

20

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 8C11 antibody is SEQ ID NO: 102, and the amino acid sequence is SEQ ID NO: 103. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 8C11 antibody are SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, respectively.

5 The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 8C11 antibody (457 bp) [Upper case: mouse 8C11VL variable region, lower case: mouse Igk light chain constant region]

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCTGCTTCCAGCAGTGATGTTGTGATGACCCAACTCC
ACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCACATCTAGTCAGACCCTTGTACACAGTAATGGAA
ACACCTATTTACATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCT
GGGGTCCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGATTTCACTCAAGATCAGCAGAGTGGAGGCTGAGGATCT
GGGAGTTTATTTCTGCTCTCACAGTACACATGTTCCATTACGTTTCGGCTCGGGGACAAAGTTGGAAATAAAacgggctg
atgctgcaccaactgtatccatcttcccaccatccagtgagcagttaacatctggag

10 The amino acid sequence of the light chain variable region of the mouse 8C11 antibody (152 a.a.) [Upper case: mouse 8C11VL variable region, lower case: mouse Igk light chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MKLPVRLLVLMFWIPASSSDVVMTQTPLSLPVSLGDQASISCTSSQTLVHSNGNTYLH
WYLQKPGQSPKLLIYKVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSHST
HVPFTFGSGTKLEIKradaaptvsifppsseqltsg

CDR1 of the light chain variable region of 8C11 antibody

TSSQTLVHSNGNTYLH

CDR2 of the light chain variable region of 8C11 antibody

15 KVSNRFS

CDR3 of the light chain variable region of 8C11 antibody

HSTHVP

20 6. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 10C3 antibody is SEQ ID NO: 84, and the amino acid sequence is SEQ ID NO: 85. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 10C3 antibody are SEQ ID NO: 26, SEQ ID NO: 27, and SEQ ID NO: 28, respectively.

The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 10C3 antibody (450 bp) [Upper case: mouse 10C3VH variable region, lower case: mouse IgG2a heavy chain constant region]

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGGCCTCATTTTAAAAGGTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGG
GGGAGACTTAGTGAGGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTGAGTTTCAGTAGCTATGGCATGT
CTTGGTTTCGCCAGACTCCAGACAAGAGGCTGGAGTGGGTCGCAACCATTAGTAGTGGTGGTAGTTACATCTACTATCCA
GAAAGTGTGAAGGGGCGATTACCATCTCCAGAGACAATGCCAGGAACATCCTGTACCTGCAAATGAGCAGTCTGAAGTC
TGAGGACACAGCCATGTATTATTGTGTAAGACTCTACGGTGGTAGGAGAGGCTATGGTTTGGACTACTGGGGTCAAGGAA
CCTCAGTCACCGTCTCCTCagccaaaacaacagccccatcggtctatcca

5 The amino acid sequence of the heavy chain variable region of the mouse 10C3 antibody (150 a.a.) [Upper case: mouse 10C3VH variable region, lower case: mouse IgG2a heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MNFGSLIFLALILKGVQCEVQLVESGGDLVRPGGSLKLSCAASGFSFSSYGMSWFRQ
TPDKRLEWVATISSGGSYIYPESVKGRFTISRDNARNILYLQMSSLKSEDTAMYYCV
RLYGGRRGYGLDYWGQGTSVTVSSakttapsvyp

CDR1 of the heavy chain variable region of 10C3 antibody
SYGMS

10 CDR2 of the heavy chain variable region of 10C3 antibody
TISSGGSYIYPESVKG

CDR3 of the heavy chain variable region of 10C3 antibody
LYGGRRGYGLDY

15 The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 10C3 antibody is SEQ ID NO: 104, and the amino acid sequence is SEQ ID NO: 105. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 10C3 antibody are SEQ ID NO: 29, SEQ ID NO: 30, and SEQ ID NO: 31, respectively.

20 The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 10C3 antibody (423 bp) [Upper case: mouse 10C3VL variable region, lower case: mouse Igk light chain constant region]

ATGAGGTTCTCTGCTCAGCTTCTGGGGCTGCTTGTGCTCTGGATCCCTGGATCCACTGCGGAAATTGTGATGACGCAGGC
 TGCATTCTCCAATCCAGTCACTCTTGGAACATCAGCTTCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTACATAGTGATG
 GCATCACTTATTGTATTGGTATCTGCAGAAGCCAGGCCAGTCTCCTCAGCTCCTGATTTATCAGATGTCCAACCTTGCC
 TCAGGAGTCCCAGACAGGTTTCAGTAGCAGTGGGTCAGGAAGTGAATTCACACTGAGAATCAGCAGAGTGGAGGCTGAGGA
 TGTGGGTGTTTATTACTGTGCTCAAAATCTAGAACTTTACACGTTCCGAGGGGGGACCAAGCTGGAAATAAAAcgggctg
 atgctgcaccaactgtatccatc

The amino acid sequence of the light chain variable region of the mouse 10C3 antibody (141 a.a.) [Upper case: mouse 10C3VL variable region, lower case: mouse Igk light chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MRFSAQLLGLLVLPWPGSTAEIVMTQAAFSNPVTLGTSASISCRSSKSLHSDGITYLY
WYLQKPGQSPQLLIYQMSNLAGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQN
LELYTFGGGTKLEIKradaaptvsi

- 5 CDR1 of the light chain variable region of 10C3 antibody
 RSSKSLHSDGITYLY
 CDR2 of the light chain variable region of 10C3 antibody
 QMSNLAG
 CDR3 of the light chain variable region of 10C3 antibody
 10 AQNLEL

7. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 11D10 antibody is SEQ ID NO: 86, and the amino acid sequence is SEQ ID NO: 87. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 11D10 antibody are SEQ ID NO: 26, SEQ ID NO: 27, and SEQ ID NO: 28,
 15 respectively.

The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 11D10 antibody (450 bp) [Upper case: mouse 11D10VH variable region, lower case: mouse IgG2b heavy chain constant region]

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGCCCTCATTTTAAAAGGTGTCCAGTGTGAGGTGCAGCTGGTGGAGTCTGG
 GGGAGACTTAGTGAGGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTTCAGTTTCAGTAGCTATGGCATGT
 CTTGGTTTCGCCAGACTCCAGACAAGAGGCTGGAGTGGGTGCAACCATTAGTAGTGGTGGTAGTTACATCTACTATCCA
 GAAAGTGTGAAGGGGCGATTACCATCTCCAGAGACAATGCCAGGAACATCCTGTACCTGCAAATGAGCAGTCTGAAGTC
 TGAGGACACAGCCATGTATTATTGTGTAAGACTCTACGGTGGTAGGAGAGGCTATGGTTTGGACTACTGGGGTCAAGGAA
 CCTCAGTCACCGTCTCCTCagccaaaacaacacccccatcagtcatacca

The amino acid sequence of the heavy chain variable region of the mouse 11D10 antibody (150 a.a.) [Upper case: mouse 11D10VH variable region, lower case: mouse IgG2b heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MNFGLSLIFLALILKGVOCEVQLVESGGDLVRPGGSLKLSCAASGFSFSSYGMSWFRQ
TPDKRLEWVATISSGGSYIYPESVKGRFTISRDNARNILYLQMSSSLKSEDTAMYYCV
RLYGGRRGYGLDYWGQGTSVTVSS akttppsvyp

5 CDR1 of the heavy chain variable region of 11D10 antibody
 SYGMS
 CDR2 of the heavy chain variable region of 11D10 antibody
 TISSGGSYIYPESVKG
 CDR3 of the heavy chain variable region of 11D10 antibody
 10 LYGGRRGYGLDY

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 11D10 antibody is SEQ ID NO: 106, and the amino acid sequence is SEQ ID NO: 107. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 11D10 antibody are SEQ ID NO: 29, SEQ ID NO: 30, and SEQ ID NO: 31,
 15 respectively.

The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 11D10 antibody (423 bp) [Upper case: mouse 11D10VL variable region, lower case: mouse Igk light chain constant region]

ATGAGGTTCTCTGCTCAGCTTCTGGGGCTGCTTGTGCTCTGGATCCCTGGATCCACTGCGGAAATTGTGATGACGCAGGC
 TGCATTCTCCAATCCAGTCACTCTTGGAAACATCAGCTTCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTACATAGTGATG
 GCATCACTTATTTGTATTGGTATCTGCAGAAGCCAGGCCAGTCTCCTCAGCTCCTGATTTATCAGATGTCCAACCTTGCC
 TCAGGAGTCCCAGACAGGTTCACTAGCAGTGGGTCAGGAACTGATTTCACTGAGAATCAGCAGAGTGGAGGCTGAGGA
 TGTGGGTGTTTATTACTGTGCTCAAAATCTAGAACTTTACACGTTCCGAGGGGGGACCAAGCTGGAAATAAAacgggctg
 atgctgcaccaactgtatccatc

The amino acid sequence of the light chain variable region (141 a.a.) of the mouse
 20 11D10 antibody [Upper case: mouse 11D10VL variable region, lower case: mouse Igk light
 chain constant region]. The underlined sequence represents a signal sequence, and the double
 underline represents CDR regions (CDR1, CDR2, and CDR3).

MRFSAQLLGLLVLPWGSTAIEIVMTQAAFSNPVTLGTSASISCRSSKSLHSDGITYLY
WYLQKPGQSPQLLIYQMSNLAGVDPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQN
LELYTFGGGTGLEIKradaaptvsi

CDR1 of the light chain variable region of 11D10 antibody

RSSKSLHSDGITYLY

CDR2 of the light chain variable region of 11D10 antibody

QMSNLAG

5

CDR3 of the light chain variable region of 11D10 antibody

AQNLEL

8. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 13D4 antibody is SEQ ID NO: 88, and the amino acid sequence is SEQ ID NO: 89. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 13D4 antibody are SEQ ID NO: 32, SEQ ID NO: 33, and SEQ ID NO: 34, respectively.

The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 13D4 antibody (472 bp) [Upper case: mouse 13D4VH variable region, lower case: mouse IgG2b heavy chain constant region]

ATGAAAGTGTGAGTCTGTTGTACCTGTTGACAGCCATTCTGGTATCCTGTCTGATGTACAGCTTCAGGAGTCAGGACC
TGGCCTCGTGAAACCTTCTCAATCTCTGTCTCTCACCTGCTCTGTCACTGGCTACTCCATCACCAGTCATTATTACTGGA
CCTGGATCCGGCAGTTTCCAGGAAACAACTGGAATGGATGGGCTACATAAGCTACGACGGTAGCAATAACTACAACCCA
TCTCTCAAAAATCGAATCTCCATCACTCGTGACACATCTAAGAACCAGTTTTTCTGAAGTTGAATTCTGTGACTACTGA
GGACACAGCTACATATACTGTGCAAGAGAGGGCCGCTCTACTATGGTAACCCCTACTGGTATTTTCGATGTCTGGGGCG
CAGGGACCAAGGTCACCGTCTCCTCagccaaaacaacacccccatcagtcctatccactggccccctaaaggcg

15

The amino acid sequence of the heavy chain variable region of the mouse 13D4 antibody (157 a.a.) [Upper case: mouse 13D4VH variable region, lower case: mouse IgG2b heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MKVLSLLYLLTAIPGILSDVQLQESGPGLVKPSQSLSLTCSVTGYSITSHYYWTWIRQF
PGNKLEWMGYISYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYNCAR
EGPLYYGNPYWYFDVWGAGTTVTVSSaktppsvyplapkg

CDR1 of the heavy chain variable region of 13D4 antibody

SHYYWT

20

75

CDR2 of the heavy chain variable region of 13D4 antibody

YISYDGSNNYNPSLKN

CDR3 of the heavy chain variable region of 13D4 antibody

EGPLYYGNPYWYFDV

5 The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 13D4 antibody is SEQ ID NO: 108, and the amino acid sequence is SEQ ID NO: 109. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 13D4 antibody are SEQ ID NO: 35, SEQ ID NO: 36, and SEQ ID NO: 37, respectively.

10 The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 13D4 antibody (404 bp) [Upper case: mouse 13D4VL variable region, lower case: mouse Igk light chain constant region]

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGATATCCAGATGACACAGAC
TACATCCTCCCTGTCTGCCTCTCTGGGGGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGACATTGACAATTATTTAA
ACTGGTATCAGCAGAAACCAGATGGAACCTGTTAACTCCTGATCTACTACACATCAAGATTACACTCAGGAGTECCATCA
AGGTTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATGTTGCCACTTACTT
TTGCCAGCAGTTTAATACGCTTCCTCGGACGTTCCGGTGGAGGCACCAAACCTGGAAATCAAacgggctgatgctgcaccaa
ctgt

The amino acid sequence of the light chain variable region of the mouse 13D4 antibody (134 a.a.) [Upper case: mouse 13D4VL variable region, lower case: mouse Igk light chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

15

MMSSAQFLGLLLLCFQGTRCDIQMTQTSSLSASLGDRVTISCRASQDIDNYLNWYQ
QKPDGTVKLLIYYTSRLHSGVPSRFSSGSGTDYSLTISNLEQEDVATYFCQQFNTLPR
TFGGG**TKLEIK**radaapt

CDR1 of the light chain variable region of 13D4 antibody

RASQDIDNYLN

CDR2 of the light chain variable region of 13D4 antibody

YTSRLHS

CDR3 of the light chain variable region of 13D4 antibody

QQFNTLP

20

9. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 13H11 antibody is SEQ ID NO: 90, and the amino acid sequence is SEQ ID NO: 91. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 13H11 antibody are SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40, respectively.

The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 13H11 antibody (471 bp) [Upper case: mouse 13H11VH variable region, lower case: mouse IgG2b heavy chain constant region]

ATGAAAGTGTGAGTCTGTTGTACCTGTTGACAGCCATTCTGGTATCCTGTCTGATGTACAGCTTCAGGAGTCAGGACC
TGGCCTCGTGAAACCTTCTCAGTCTCTGTCTCTCACCTGCTCTGTCACTGGCTACTCCATCTCCAGTCATTATTACTGGA
GTTGGATCCGGCAGTTTCCAGGAAACAGACTGGAATGGATGGGCTACATAAGCTACGACGGTAGCAATAACTACAACCCA
TCTCTCAAAAATCGAATCTCCATCACTCGTGACACATCTAAGAACCAGTTTTTCTGAAGTTGAATTCTGTGACTACTGA
GGACACAGCTACATATAACTGTGCAAGAGAGGGCCCGCTCTACTATGGTAACCCCTACTGGTATTTTCGATGTCTGGGGCG
CAGGGACCACGGTCACCGTCTCCTCagccaaaacaacaccccccatcagtcctatccactggccccctaagggc

The amino acid sequence of the heavy chain variable region of the mouse 13H11 antibody (157 a.a.) [Upper case: mouse 13H11VH variable region, lower case: mouse IgG2b heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MKVLSLLYLLTAIPGILSDVQLQESGPLVKPSQSLSLTCSVTGYSISSHYYWSWIRQF
PGNRLEWMGYISYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYNCARE
GPLYYGNPYWYFDVWGAGTTVTVSSaktppsvyplapkg

CDR1 of the heavy chain variable region of the 13H11 antibody
SHYYWS

15 CDR2 of the heavy chain variable region of the 13H11 antibody
YISYDGSNNYNPSLKN

CDR3 of the heavy chain variable region of the 13H11 antibody
EGPLYYG

20 The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 13H11 antibody is SEQ ID NO: 110, and the amino acid sequence is SEQ ID NO: 111. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 13H11 antibody are SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43, respectively.

The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 13H11 antibody (414 bp) [Upper case: mouse 13H11VL variable region, lower case: mouse Igk light chain constant region]

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGATATCCAGATGACACAGAC
TACATCCTCCCTGTCTGCCTCTCTGGGGGGCAGCGTCACCATCAGTTGCAGGGCAAGTCAGGACATTGACAATTATTTAA
ACTGGTATCAGCAAAAACCAGATGGAACCTGTTAACTCCTGATCTACTACACATCAAGATTACACTCAGGAGTCCCATCA
AGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAACAAGAAGATATTGCCACTTACTT
TTGCCAACAGTTTAATACGCTTCCTCGGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAAACgggctgatgctgcaccaa
ctgtatccatcttc

5 The amino acid sequence of the light chain variable region of the mouse 13H11 antibody (138 a.a.) [Upper case: mouse 13H11VL variable region, lower case: mouse Igk light chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MMSSAQFLGLLLLCFQGTRCDIQMTQTSSLSASLGGSVTISCRASQDIDNYLNWYQ
QKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQFNTLPR
TFGGGTKLEIKradaaptvsif

CDR1 of the light chain variable region of the 13H11 antibody

RASQDIDNYLN

10 CDR2 of the light chain variable region of the 13H11 antibody

YTSRLHS

CDR3 of the light chain variable region of the 13H11 antibody

QQFNTLP

15 10. The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 14C1 antibody is SEQ ID NO: 92, and the amino acid sequence is SEQ ID NO: 93. The amino acid sequences of CDR1, CDR2, and CDR3 in the heavy chain variable region of the mouse 14C1 antibody are SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40, respectively.

20 The nucleic acid sequence of the heavy chain variable region of anti-PLD4 mouse 14C1 antibody (470 bp) [Upper case: mouse 14C1VH variable region, lower case: mouse IgG1 heavy chain constant region]

ATGAAAGTGTGAGTCTGTTGTACCTGTTGACAGCCATTCCTGGTATCCTGTCTGATGTACAGCTTCAGGAGTCAGGACC
 TGGCCTCGTGAAACCTTCTCAGTCTCTGTCTCTCACCTGCTCTGTCACTGGCTACTCCATCTCCAGTCATTATTACTGGA
 GTTGGATCCGGCAGTTTCCAGGAAACAGACTGGAATGGATGGGCTACATAAGCTACGACGGTAGCAATAACTACAACCCA
 TCTCTCAAAAATCGAATCTCCATCACTCGTGACACATCTAAGAACCAGTTTTTCTGAAGTTGAATTCTGTGACTACTGA
 GGACACAGCTACATATAACTGTGCAAGAGAGGGCCCGCTCTACTATGGTAACCCCTACTGGTATTTTCGATGTCTGGGGCG
 CAGGGACCACGGTCACCGTCTCCTCagccaaaacgacacccccatctgtctatccactggccccctaaggg

The amino acid sequence of the heavy chain variable region of the mouse 14C1 antibody (156 a.a.) [Upper case: mouse 14C1VH variable region, lower case: mouse IgG1 heavy chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MKVLSLLYLLTAIPGILSDVQLQESGPGLVKPSQSLSLTCSVTGYSISSHYYWSWIRQF
 PGNRLEWMGYISYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYNCARE
GPLYYGNPYWYFDVWGAGTTVTVSS aktppsvyplapk

- 5 CDR1 of the heavy chain variable region of 14C1 antibody
 SHYYWS
 CDR2 of the heavy chain variable region of 14C1 antibody
 YISYDGSNNYNPSLKN
 CDR3 of the heavy chain variable region of 14C1 antibody
 10 EGPLYYGNPYWYFDV

- The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 14C1 antibody is SEQ ID NO: 112, and the amino acid sequence is SEQ ID NO: 113. The amino acid sequences of CDR1, CDR2, and CDR3 in the light chain variable region of the mouse 14C1 antibody are SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43,
 15 respectively.

The nucleic acid sequence of the light chain variable region of anti-PLD4 mouse 14C1 antibody (465 bp) [Upper case: mouse 14C1VL variable region, lower case: mouse Igk light chain constant region]

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGATATCCAGATGACACAGAC
 TACATCCTCCCTGTCTGCCTCTCTGGGGGGCAGCGTCACCATCAGTTGCAGGGCAAGTCAGGACATTGACAATTATTTAA
 ACTGGTATCAGCAAAAACCAGATGGAAGTGTAACTCCTGATCTACTACACATCAAGATTAACTCAGGAGTCCCATCA
 AGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAACAAGAAGATATTGCCACTTACTT
 TTGCCAACAGTTTAATACGCTTCCTCGGACGTTGGGTGGAGGCACCAAGCTGGAAATCAAacgggctgatgctgcaccaa
 ctgtatccatcttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgtgttc

The amino acid sequence of the light chain variable region of the mouse 14C1 antibody (155 a.a.) [Upper case: mouse 14C1VL variable region, lower case: mouse Igk light chain constant region]. The underlined sequence represents a signal sequence, and the double underline represents CDR regions (CDR1, CDR2, and CDR3).

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGGSVTISCRASQDIDNYLNWYQ
QKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQFNTLP
 TFGGGTKLEIKradaaptvsifppsseqltsggasvvcf

5 CDR1 of the light chain variable region of 14C1 antibody

RASQDIDNYLN

CDR2 of the light chain variable region of 14C1 antibody

YTSRLHS

CDR3 of the light chain variable region of 14C1 antibody

10 QQFNTLP

[0138]

The base sequences and the amino acid sequences of the heavy chain and the light chain of the prepared chimeric 11G9 antibody are following Sequence Nos., respectively.

Heavy chain

15 SEQ ID NO: 120 (base sequence)

SEQ ID NO: 121 (amino acid sequence)

Light chain

SEQ ID NO: 122 (base sequence)

SEQ ID NO: 123 (amino acid sequence)

20 [0139]

11. The nucleic acid sequence of the heavy chain of anti-PLD4 chimeric 11G9 antibody (1401 bp) [Upper case: chimeric 11G9VH variable region, lower case: human IgG1 heavy chain constant region] (SEQ ID NO: 120)

ATGAAAGTGTGAGTCTGTTGTACCTGTTGACAGCCATTCTGGTATCCTGTCTcagGTCCAAGTGCAGCAGCCTGGGGC
TGAAGTGGTGAAGCCTGGGACTTCAGTGAAGATGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTGGATGCACT
GGGTGAAGCAGAGGCCGGGACAAGGCCTTGAGTGGATTGGAGATATTTATCCTGGTAGTAGTACTAATACTACAATGAG
AAGTTCAAGAGCAAGGCCACACTGACTGTAGACACATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTGACATCTGA
GGACTCTGCGGTCTATTACTGTGCAAGAGGAGGGTGGTTGGATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCG
TCTCCTCagctagcaccaagggcccatcggtcttccccctggcaccctcctccaagagcaccctctgggggcacagcgcc
ctgggctgcctgggtcaaggactacttccccgaaccgggtgacgggtgctgtggaactcaggcgccctgaccagcgccgtgca
caccctccccggctgtcctacagtccctcaggactctactccctcagcagcggtgggtgaccgtgccccccagcagcttgggca
cccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaattcttgtgac
aaaactcacacatgcccaccgtgcccagcaccctgaactcctggggggaccgtcagctcttcttccccccaaaacccaa
ggacaccctcatgatctccccggacccttgaggtcacatgcgtgggtgggtggacgtgagccacgaagaccctgaggtcaagt
tcaactggtaactggacggcggtggaggtgcataatgccaaagacaaagccgcgggaggagcagtaaacagcagctaccgt
gtggctcagcgctctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccct
cccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatccc
gggatgagctgaccaagaaccaggctcagcctgacctgctgggtcaaaggttctatcccagcgacatcgccgtggagtgg
gagagcaatgggcagccggagaaacaactacaagaccagccctcccgctgctggactccgacggctccttcttctctacag
caagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaacc
actacagcgagaagagcctctccctgtctccgggtaaatga

[0140]

12. The amino acid sequence of the heavy chain of anti-PLD4 chimeric 11G9 antibody (466 a.a.) [Upper case: chimeric 11G9VH variable region, lower case: human IgG1 heavy chain constant region] (SEQ ID NO: 121)

MKVLSELLYLLTAIPGILSQVQLQQPGAELVKPGTSVKMSCKASGYTFSTSYWMHWVKQRPGQGLEWIGDIYPGSDSTNYNE
KFKSKATLTVDTSSTAYMQLSSLTSEDSAVYYCARGGWLDAMDYWGQGTSTVTVSSastkgpsvflapsskstsggtaa
lgclvkdyfpepvtvswngaltsgvhtfpavllqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkvepkscd
kthtcppcpapelggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltknqvslctclvkgyfypsdiavew
esngqpennyktlppvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqkslsispkg

5 [0141]

13. The nucleic acid sequence of the light chain of anti-PLD4 chimeric 11G9 antibody (705 bp) [Upper case: chimeric 11G9VL variable region, lower case: human Igk light chain constant region] (SEQ ID NO: 122)

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATGTGATATCCAGATGACACAGAC
TACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGACATTAGCAATTATTTAA
ACTGGTATCAGCAGAAACCAGATGGAAGTGTAAACTCCTGATCTACTACACATCAAGATTACACTCAGGAGTCCCATCA
AGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTT
TTGCCAACAGGGTAATACGCTTCCGTGGACGTTCCGGTGGAGGCACCAAGCTGGAAATCAAacgaactgtggctgcaccat
ctgtcttcatcttccccccatctgatgagcagttgaaatctggaactgccctctgttgtgtgcctgctgaataacttctat
cccagagaggccaaagtacagtggaaagggtgataacgccccccaatcgggtaactcccaggagagtgctcacagagcagga
cagcaaggacagcaccctacagcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagctctacgctt
gcgaagtcacccatcagggcctgagctcgccccgtcacaaagagcttcaacaggggagagtgctag

[0142]

14. The amino acid sequence of the light chain of anti-PLD4 chimeric 11G9 antibody (234 a.a.) [Upper case: chimeric 11G9VL variable region, lower case: human Igk light chain constant region] (SEQ ID NO: 123)

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQOKPDGTVKLLIYYTSRLHSGVPS
RFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGGGKLEIKrtvaapsvfi fppsdeqlksgtasvvc llnfy
preakvqwkvdnalqsgnsqesvteqdsksdyslsstl lskadyekhkvyacevthqglsspvtksfnrgec

5 [0143]

Sequence of cDNA and protein in PLD4-related molecular

> Human PLD4 cDNA (1521 bp) (SEQ ID NO: 44)

ATGCTGAAGCCTCTTTGGAAAGCAGCAGTGGCCCCACATGGCCATGCTCCATGCCGCCCCGCCGCCCGTGGGACAGAGA
GGCTGGCACGTTGCAGGTCTGGGAGCGCTGGCTGTGCTGTGGCTGGGCTCCGTGGCTCTTATCTGCCTCCTGTGGCAAG
TGCCCCGTCTCCACCTGGGGCCAGGTGCAGCCCAAGGACGTGCCAGGTCTGGGAGCATGGCTCCAGCCCAGCTTGG
GAGCCCCCTGGAAGCAGAGGCCAGGCAGCAGAGGGACTCCTGCCAGCTTGTCCTTGTGGAAAGCATCCCCAGGACCTGCC
ATCTGCAGCCGGCAGCCCCCTCTGCCAGCCTCTGGGCCAGGCCTGGCTGCAGCTGCTGGACACTGCCAGGAGAGCGTCC
ACGTGGCTTCATACTACTGGTCCCTCACAGGGCCTGACATCGGGGTCAACGACTCGTCTTCCAGCTGGGAGAGGCTCTT
CTGCAGAAGCTGCAGCAGCTGCTGGGCAGGAACATTTCCCTGGCTGTGGCCACCAGCAGCCCGACACTGGCCAGGACATC
CACCGACCTGCAGGTTCTGGCTGCCCCAGGTGCCCATGTACGACAGGTGCCCATGGGGCGGCTCACCAGGGGTGTTTTGC
ACTCCAAATTCTGGGTTGTGGATGGACGGCACATATACATGGGCAGTGCCAACATGGACTGGCGGTCTCTGACGCAGGTG
AAGGAGCTTGGCGCTGTCATCTATAACTGCAGCCACCTGGCCCAAGACCTGGAGAAGACCTTCCAGACCTACTGGGTACT
GGGGGTGCCCAAGGCTGTCTCCCCAAAACCTGGCCTCAGAACTTCTCATCTCACTTCAACCGTTTCCAGCCCTTCCACG
GCCTCTTTGATGGGGTGCCCACTGCTACTTCTCAGCGTCGCCACCAGCACTCTGTCCCAGGGCCGCACCCGGGAC
CTGGAGGCGCTGCTGGCGGTGATGGGGAGCGCCAGGAGTTCATCTATGCCTCCGTGATGGAGTATTTCCCACACGCG
CTTCAGCCACCCCCGAGGTACTGGCCGGTGCTGGACAACGCGCTGCGGGCGGACGCTTCGGCAAGGGCGTGCGCGTGC
GCCTGCTGGTGGCTGCGGACTCAACACGGACCCACCATGTTCCCCTACCTGCGGTCCCTGCAGGCGCTCAGCAACCCC
GCGGCCAACGTCTCTGTGGACGTGAAAGTCTTCATCGTGCCGGTGGGGAACCATTCACATCCCATTCAGCAGGGTGAA
CCACAGCAAGTTCATGGTCACGGAGAAGGCAGCCTACATAGGCACCTCCAACCTGGTCCGAGGATTACTTCAGCAGCACGG
CGGGGGTGGGCTTGGTGGTCACCCAGAGCCCTGGCGCGCAGCCCGCGGGGGCCACGGTGCAGGAGCAGCTGCGGCAGCTC
TTTGAGCGGACTGGAGTTCGCGCTACGCCGTGGCCTGGACGGACAGGCTCCGGGCCAGGACTGCGTTTGGCAGGGCTG
A

> Human PLD4 protein (506 amino acids) (SEQ ID NO: 1)

MLKPLWKA AVAPTWP CSMPPRRP WDREAGTLQVLGALAVLWLGSVALICLLWQVPRPPTWGQVQPKDVPR
SWEHGSSPAWEPLAEARQQRDSCQLVLVESIPQDLPSAAGSPSAQPLGQAWLQLLDTAQESVHVASYW
SLTGPDIGVNDSSSQLGEALLQKLQQLGRNISLAVATSSPTLARTSTDLQVLAARGAHVRQVPMGRLTR
GVLHSKFWVDGRHIYMG SANMDWRS LTQVKELGAVIYNCSHLAQDLEKTFQTYWVLGVPKAVLPKTPWQ
NFSSHFNRFQPFHGLFDGVPTTAYFSASPPALCPQGRTRDLEALLAVMGS AQEF IYASVMEYFPTTRFSH
PPRYWPVLDNALRAAAF GKGVRRVRL LVGCGLNTDPTMFPYLRSLQALSNPAANVSVDVKVFIVPVGNHSN
IPFSRVNH SKFMVTEKAAYIGTSN WSEDFSS TAGVGLVVTQSPGAQPAGATVQEQLRQLFERDWSSRYA
VGLDGQAPGQDCVWQG

> cynomolgus monkey PLD4 cDNA (1521 bp) (SEQ ID NO: 63)

ATGCTGAAGCCTCTTCGGAGAGCgGCAGTGACCCCCATGTGGCCGTGCTCCATGCTGCCCCGCCGCCTGTGGGACAGAGA
GGCTGGCAGGTTGCAGGTCTTGGGAGTGCTGGCTATGCTGTGGCTGGGCTCCATGGCTCTTACCTACCTCCTGTGGCAAG
TGCGCCGTCCTCCACCTGGGGCCAGGTGCAGCCCAAGGACGTGCCCAGGTCTTGGGGGCATGGTTCCAGCCCAGCTCTG
GAGCCCCTGGAAGCGGAGGTCAGGAAGCAGAGGGACTCCTGCCAGCTTGTCTTGTGGAAAGCATCCCCCAGGACCTGCC
ATTTGCAGCCGGCAGCCTCTCCGCCAGCCTCTGGGCCAGGCCTGGCTGCAGCTGCTGGACACTGCCCAGGAGAGCGTCC
ACGTGGCTTCATACTACTGGTCCCTCACAGGGCCCGACATTGGGGTCAACGACTCATCTTCCAGCTGGGAGAGGCCCTT
CTGCAGAAGCTGCAGCAGCTGCTGGGCAGGAACATTTCTTGGCTGTGGCCACCAGCAGTCCAACACTGGCCAGGAAGTC
CACCGACCTGCAGGTCTTGGCTGCCCCAGGTGCCCAGGTACGACGGGTGCCCATGGGGCGGCTCACCAGGGGCGTTTTGC
ACTCCAAATTCTGGGTGTGGATGGACgGCACATATACATGGGCAGTGCcAACATGGACTGGCGGTCCCTGACGCAGGTG
AAGGAGCTTGGCGCTGTCTATAACTGCAGCCACCTGGCCCAAGACCTGGAGAAGACCTTCCAGACCTACTGGGTGCT
GGGGGTGCCCAAGGCTGTCTCCCCAAAACCTGGCCTCAGAACTTCTCATCTCACATCAACCGTTTCCAGCCCTTCCAGG
GCCTCTTTGATGGGGTGGCCACCACTGCCTACTTCTCAGCATCGCCACCcGCACTCTGTCCCAGGGCCGCACCCCTGAC
CTGGAGGCGCTGTTGGCGGTGATGGGGAGCGCCAGGAGTTCATCTATGCCTCCGTGATGGAGTATTTCCCTACCACgCG
CTTCAGCCACCCCCCGCAGGTACTGGCCGGTGCTGGACAACCGCTGCGGGCGGCAGCCTTCAGCAAGGGTGTGCGCGTGC
GCCTGCTGGTCAGCTGCGGACTCAACACGGACCCCAACATGTTCCCTATCTGCGGTCCCTGCAGGCGCTCAGCAACCCC
GCGGCCAACGTCTCTGTGGACGTGAAAGTCTTCATCGTGCCGGTGGGGAATCATTCCAACATCCCGTTCAGCAGGGTGAA
CCACAGCAAGTTCATGGTCACGGAGAAGGCAGCCTACATAGGCACCTCCAACCTGGTCGGAGGATTACTTCAGCAGCAGCA
CGGGGGTGGGCCTGGTGGTCACCCAGAGCCCCGGCGCGCAGCCCGCGGGGGCCACGGTACAGGAGCAGCTGCGGCAGCTC
TTTGAGCGGGACTGGAGTTCGCGCTACGCCGTGGCCTGGACGGACAGGCTCCGGGCCAGGACTGCGTTTGGCAGGGCTG
A

> cynomolgus monkey PLD4 protein (506 amino acids) (SEQ ID NO: 129)

MLKPLRRAAVTPMWPCSMPLPRRLWDREAGTLQVLGVLAMLWLGSMAITYLLWQVRRPPTWGQVQPKDVPRSWGHSPPAL
EPLAEVRKQRDSCQLVLYESIPQDLPAAGSLSAQPLGQAWLQLLDTAQESVHVASYYSWLTGPDIGVNDSSSQLGEAL
LQKLQQLLGRNISLAVATSSPTLARKSTDQVLAARGAQVRRVPMGRLTRGVLHSEFWVVDGRHIYMGSANMDWRSITQV
KELGAVIYNCSHLAQDLEKTFQTYWVLGVPKAVLPKTWPQNFSSHINRFQPFQGLFDGVPTTAYFSASPPALCPQGRTPD
LEALLAVMGSAQEFIYASVMEYFPTTRFSHPRRYWPVLDNALRAAAFSGKVRVRLLVSCGLNTDPTMFPYLRSLQALSNP
AANVSVDVKVFIYPVGNHSNIPFSRVNHSKFMVTEKAAYIGTSNWSSEDFSSITGVGLVVTQSPGAQPAGATVQEQLRQL
FERDWSSRYAVGLDQAPGQDCVWQG

> rhesus monkey PLD4 cDNA (1521 bp) (SEQ ID NO: 124)

ATGCTGAAGCCTCTTCGGAGAGCGGCAGTGACCCCCATGTGGCCGTGCTCCATGCTGCCCCGCCGCTGTGGGACAGAGA
 GGCTGGCACGTTGCAGGTCTGGGAGTGCTGGCTATGCTGTGGCTGGGCTCCATGGCTCTTACCTACCTCCTGTGGCAAG
 TGGCTGTCTCCACCTGGGGCCAGGTGCAGCCAGGGACGTGCCAGGTCTGGGGGCATGGTTCCAGCCTAGCTCTG
 GAGCCCCCTGGAAGCGGAGGTCAGGAAGCAGAGGGACTCCTGCCAGCTTGTCCTTGTGGAAAGCATCCCCAGGACCTGCC
 ATTTGCAGCCGGCAGCCTCTCCGCCAGCCTCTGGGCCAGGCCTGGCTGCAGCTGCTGGACACTGCCCAGGAGAGCGTCC
 ACGTGGCTTCATACTACTGGTCCCTCACAGGGCCCCGACATTGGGGTCAACGACTCATCTTCCAGCTGGGAGAGGCCCTT
 CTGCAGAAGCTGCAGCAGCTGCTGGGCAGGAACATTTCTTGGCTGTGGCCACCAGCAGTCCAACACTGGCCAGGAAGTC
 CACCGACCTGCAGGTCTGGCTGCCCGAGGTGCCCAGGTACGACGGGTGCCCATGGGGCGGCTCACCAGGGGCGTTTTGC
 ACTCCAAATTCTGGGTGTGGATGGACGGCACATATACATGGGCAGTGCCAACATGGACTGGCGGTCCCTGACGCAGGTG
 AAGGAGCTTGGCGCTGTCATCTATAACTGCAGCCACCTGGCCCAAGACCTGGAGAAGACCTTCCAGACCTACTGGGTGCT
 GGGGGTGCCCAAGGCTGTCCTCCCCAAAACCTGGCCTCAGAACTTCTCATCTCACATCAACCGTTTCCAGCCCTTCCAGG
 GCCTCTTTGATGGGGTGCCCACTGCCTACTTCTCAGCATCGCCACCCGCACTCTGTCCCCAGGGCCGCACCCCTGAC
 CTGGAGGCGCTGTTGGCGGTGATGGGGAGCGCCCAGGAGTTCATCTATGCCTCCGTGATGGAGTATTTCCCTACCACGCG
 CTTCAGCCACCCCCGCAGGTACTGGCCGGTGCTGGACAACGCGCTGCGGGCGGCAGCCTTCAGCAAGGGTGTGCGCGTGC
 GCCTGCTGGTCAGCTGCGGACTCAACACGGACCCACCATGTTCCCTATCTGCGGTCCCTGCAGGCGCTCAGCAACCCC
 GCGGCCAACGTCTCTGTGGACGTGAAAGTCTTCATCGTGCCGGTGGGGAATCATTCCAACATCCCGTTCAGCAGGGTGAA
 CCACAGCAAGTTCATGGTCACGGAGAAGGCAGCCTACATAGGCACCTCCAACCTGGTCGGAGGATTACTTCAGCAGCACGA
 CGGGGGTGGGCCTGGTGGTCACCCAGAGCCCCGGCGCGCAGCCGCGGGGGCCACGGTACAGGAGCAGCTGCGGCAGCTC
 TTTGAGCGGGACTGGAGTTCGCGCTACGCCGTGGCCTGGACGGACAGGCTCCGGGCCAGGACTGCGTTTGGCAGGGCTG
 A

> rhesus monkey PLD4 protein (506 amino acids) (SEQ ID NO: 130)

MLKPLRRAAVTPMWPCSMPLPRRLWDREAGTLQVLGVLAMLWLGSMAITYLLWQVRCPTTWGQVQPRDVPRSWGHS
 EPLEAEVRKQRDSCQLVLVESIPQDLFPAAGSLSAQPLGQAWLQLLDTAQESVHVASYYSWLTGPDIGVNDSSSQLGEAL
 LQKLQQLLGRNISLAVATSSPTLARKSTDQVLAARGAQVRRVPMGRLTRGVLSKFWVDGRHIYMGSA
 NMDWRS
 LTQVKELGAVIYNCSHLAQDLEKTFQTYWVLGVPKAVLPKTWPQNFSSHINRFQPFQGLFDGVPTTAYFSASPPALCPQGRTPD
 LEALLAVMGS
 AQEF
 IYASVMEYFPTTRFSHPRRYWPVLDNALRAAAF
 SKGVRVRL
 LVSCGLNTDPTMFPYLRSLQALSNP
 AANVSVDVKVFI
 VPVGNHSNIPFSRVNHSKFMVTEKAAYIGTSNWS
 EDYFSSTTGVGLVVTQSPGAQPAGATVQEQLRQL
 FERDWSSRYAVGLDGQAPGQDCVWQG

> Mouse PLD4 cDNA (1512 base pairs) (SEQ ID NO: 131)

ATGGACAAGAAGAAAGAGCAGCCAGAGATGCGGATACCACTCCAGACAGCAGTGGAGGTCTCTGATTGGCCCTGCTCCAC
 ATCTCATGATCCACATAGCGGACTTGGCATGGTACTGGGGATGCTAGCTGTACTGGGACTCAGCTCTGTGACTCTCATCT
 TGTTCCTGTGGCAAGGGGCCACTTCTTTCACCAGTCATCGGATGTTCCCTGAGGAAGTGGCCCTCCTGGTCCTGGGAGACC
 CTGAAAGGAGACGCTGAGCAGCAGAATAACTCCTGTCAGCTCATCCTTGTGGAAAGCATCCCCGAGGACTTGCCATTTGC
 AGCTGGCAGCCCCACTGCCCAGCCCCCTGGCCCAGGCTTGGCTGCAGCTTCTTGACACTGCTCGGGAGAGCGTCCACATTG
 CCTCGTACTACTGGTCCCTCACTGGACTGGACATTGGAGTCAATGACTCGTCTTCTCGGCAGGGAGAGGCCCTTCTACAG
 AAGTTCCAACAGCTTCTTCTCAGGAACATCTCTGTGGTGGTGGCCACCCACAGCCCAACATTGGCCAAGACATCCACTGA
 CCTCCAGGTCTTGGCTGCCCATGGTGGCCAGATACGACAAGTGGCCATGAAACAGCTTACTGGGGGTGTTCTACACTCCA
 AATTCTGGGTTGTGGATGGGCGACACGTCTACGTGGGCAGCGCCAACATGGACTGGCGGTCCCTGACTCAGGTGAAGGAA
 CTTGGTGCAATCATCTACAACCTGCAGCAACCTGGCTCAAGACCTTGAGAAAACATTCCAGACCTACTGGGTGCTAGGGAC
 TCCCCAAGCTGTTCTCCCTAAACCTGGCCTCGGAACTTCTCATCCACATCAACCGCTTCCATCCCTTGCGGGGTCCCT
 TTGATGGGGTTCCCAACACGGCCTATTTCTCGGCCTCCCCTCCCTCCTCTGCCCAGATGGCCGGACCCGGGATCTGGAC
 GCAGTGTGGGAGTGATGGAGGGTGCTCGCCAGTTCATCTATGTCTCGGTGATGGAGTATTTCCCTACCACGCGCTTAC
 CCACCATGCCAGGTACTGGCCCGTGCTGGACAATGCGCTACGGGCAGCGGCCCTCAATAAGGGTGTGCATGTGCGCTTAC
 TGGTCAGCTGCTGGTTCAACACAGACCCCAACATGTTGCTTATCTGAGGTCCCTGCAGGCTTTCAGTAACCCCTCGGCT
 GGCATCTCAGTGGATGTGAAAGTCTTCATCGTGCCTGTGGGAAATCATTCCAACATCCCGTTCAGCCGCGTGAACCACAG
 CAAGTTCATGGTCACAGACAAGACAGCCTATGTAGGCACCTCTAACTGGTCAGAAGACTACTTCAGCCACACCGCTGGTG
 TGGGCCTGATTGTCAGCCAGAAGACCCCCAGAGCCCAGCCAGGCGCAACCACCGTGCAGGAGCAGCTGAGGCAACTCTTT
 GAACGAGACTGGAGTTCCCACTATGCTATGGACCTAGACAGACAAGTCCCGAGCCAGGACTGTGTCTGGTAG

> Mouse PLD4 protein (503 amino acids) (SEQ ID NO: 132)

MDKKKEHPMRIPLQTAVEVSDWPCSTSHDPHSGLGMLVGLMLAVLGLSSVTLILFLWQGATSFTSHRMFPPEEVPSWSWET
 LKGDAEQNNNSQLILVESIPEDLPFAAGSPTAQPLAQAWLQLLDTARESVHIASYYWSLTGLDIGVNDSSSRQGEALLQ
 KFQQLLLRNISVVVATHSPTLAKTSTDQLVLAHGAQIRQVPMKQLTGGVLHSEFWVVDGRHVYVGSANMDWRSLSLQVKE
 LGAI IYNCSNLAQDLEKTFQTYWVLGTPQAVLPKTWPRNFSSHINRFHPLRGPFDPVPTTAYFSASPPSLCPHGRTRDLD
 AVLGVMEGARQFIYVSVMEYFPTTRFTHHARYWPVLDNALRAAALNKGHVRLLVSCWFNTDPTMFAYLRSLSLQAFSNPSA
 GISVDVKVFIYPVGNHSNIPFSRVNHSKFMVTDKTAYVGTSNWSYDYSHTAGVGLIVSQKTPRAQPGATTVQEQLRQLF
 ERDWSSHYAMDLDLDRQVPSQDCVW

> Human PLD3 cDNA sequence (SEQ ID NO: 55)

ATGAAGCCTAAACTGATGTACCAGGAGCTGAAGGTGCCTGCAGAGGAGCCCGCCAATGAGCTGCCCATGAATGAGATTGA
GGCGTGGAAGGCTGCGGAAAAGAAAGCCCGCTGGGTCTGCTGGTCCTCATTCTGGCGGTTGTGGGCTTCGGAGCCCTGA
TGA CTCAGCTGTTTCTATGGGAATACGGCGACTTG CATCTCTTTGGGCCCAACCAGCGCCAGCCCCCTGCTATGACCCT
TGCGAAGCAGTGCTGGTGGAAAGCATTCTGAGGGCCTGGACTTCCCCAATGCCTCCACGGGGAACCCTTCCACCAGCCA
GGCCTGGCTGGGCCTGCTCGCCGGTGGCGACAGCAGCCTGGACATCGCCTCCTTCTACTGGACCCTCACCAACAATGACA
CCACACGCAGGAGCCCTCTGCCAGCAGGGTGAGGAGGTCTCCGGCAGCTGCAGACCCTGGCACCAAGGGCGTG AAC
GTCCGCATCGCTGTGAGCAAGCCAGCGGGCCCCAGCCACAGGCGGACCTGCAGGCTCTGCTGCAGAGCGGTGCCCAGGT
CCGCATGGTGGACATGCAGAAGCTGACCCATGGCGTCTGCATACCAAGTTCTGGGTGGTGGACCAGACCCACTTCTACC
TGGGCAGTGCCAACATGGACTGGCGTTCCTGACCCAGGTCAAGGAGCTGGGCGTGGTCATGTACAACTGCAGCTGCCTG
GCTCGAGACCTGACCAAGATCTTTGAGGGCTACTGGTTCTGGGCCAGGCAGGCAGCTCCATCCCATCAACTTGGCCCCG
GTTCTATGACACCCGCTACAACCAAGAGACACCAATGGAGATCTGCCTCAATGGAACCCCTGCTCTGGCCTACCTGGCGA
GTGCGCCCCCACCCTGTGTCCAAGTGGCCGCACTCCAGACCTGAAGGCTCTACTCAACGTGGTGGACAATGCCCGGAGT
TTCATCTACGTCGCTGT CATGAACTACCTGCCACTCTGGAGTTCTCCACCCCTCACAGGTTCTGGCCTGCCATTGACGA
TGGGCTGCGGCGGGCCACCTACGAGCGTGGCGTCAAGGTGCGCCTGCTCATCAGCTGCTGGGGACACTCGGAGCCATCCA
TGCGGGCCTTCTGCTCTCTCTGGCTGCCCTGCGTGACAACCATACCCACTCTGACATCCAGGTGAAACTCTTTGTGGTC
CCCGCGGATGAGGCCAGGCTCGAATCCCATATGCCCGTGTCAACCACAACAAGTACATGGTGA CTGAACGCGCCACCTA
CATCGGAACCTCCA ACTGGTCTGGCAACTACTTCACGGAGACGGCGGGCACCTCGCTGCTGGTGACGCAGAATGGGAGGG
GCGGCCTGCGGAGCCAGCTGGAGGCCATTTTCTGAGGGACTGGGACTCCCCTTACAGCCATGACCTTGACACCTCAGCT
GACAGCGTGGGCAACGCTGCCGCTGCTCTGA

> Human PLD3 protein (490 amino acids) (SEQ ID NO: 127)

MKPKLMYQELKVP AE E P A N E L P M N E I E A W K A A E K K A R W V L L V L I L A V V G F G A L M T Q L F L W E Y G D L H L F G P N Q R P A P C Y D P
C E A V L V E S I P E G L D F P N A S T G N P S T S Q A W L G L L A G A H S S L D I A S F Y W T L T N N D T H T Q E P S A Q Q G E E V L R Q L Q T L A P K G V N
V R I A V S K P S G P Q P Q A D L Q A L L Q S G A Q V R M V D M Q K L T H G V L H T K F W V V D Q T H F Y L G S A N M D W R S L T Q V K E L G V V M Y N C S C L
A R D L T K I F E A Y W F L G Q A G S S I P S T W P R F Y D T R Y N Q E T P M E I C L N G T P A L A Y L A S A P P L C P S G R T P D L K A L L N V D N A R S
F I Y V A V M N Y L P T L E F S H P H R F W P A I D D G L R R A T Y E R G V K V R L L I S C W G H S E P S M R A F L L S L A A L R D N H T H S D I Q V K L F V V
P A D E A Q A R I P Y A R V N H N K Y M V T E R A T Y I G T S N W S G N Y F T E T A G T S L L V T Q N G R G G L R S Q L E A I F L R D W D S P Y S H D L D T S A
D S V G N A C R L L

> Human PLD5 cDNA (1338 base pairs) (SEQ ID NO: 56)

ATGGGAGAGGATGAGGATGGACTCTCAGAAAAAATTGCCAAAATAAATGTCGAATTGCCCTGGTGGAAAATATTCCTGA
-AGGCCTTA ACTATT CAGAAAATGCACCATTTCACTTATCACTTTTCCAAGGCTGGATGAATTTACTCAACATGGCCAAA
AGTCTGTTGACATAGTGTCTTCCCATTTGGGATCTCAACCACACTCATCCATCAGCATGTCAGGGTCAACGTCTTTTGA
AAGTTGCTCCAGCTGACTTCGCAAAATATTGAAATCAAGCTAGTGAGTGATGTAACAGCTGATTCAAAGGTATTAGAAGC
CTTGAAATTAAGGGAGCCGAGGTGACGTACATGAACATGACCGCTTACAACAAGGGCCGGCTGCAGTCCTCCTTCTGGA
TCGTGGACAAACAGCACGTGTATATCGGCAGTGCCGGTTTGGACTGGCAATCCCTGGGACAGATGAAAGAACTCGGTGTC
ATCTTCTACAACTGCAGCTGCCTGGTCCTAGATTTACAAAGGATATTTGCTCTATATAGTTCATTAATAATTCAAAGCAG
AGTGCCTCAAACCTGGTCCAAAAGACTCTATGGAGTCTATGACAATGAAAAGAAATTGCAACTTCAGTTGAATGAAACCA
AATCTCAAGCATTTGTATCGAATTCTCCAAAACCTCTTTTGGCCCTAAAAACAGAAGTTTTGACATAGATGCCATCTACAGT
GTGATAGATGATGCCAAGCAGTATGTGTACATCGCTGTCATGGACTACCTGCCTATCTCCAGCACAAGCACCAAAAGGAC
TTACTGGCCAGACTTGGATGCAAAAATAAGAGAAGCATTAGTTTTACGAAGCGTTAGAGTTCGACTCCTTTTAAGCTTCT
GGAAGGAACTGATCCCCTTACGTTTAACTTTATTTCACTCTTAAAGCGATTTGCACTGAAATAGCCAACTGCAGTTTG
AAAGTTAAATTTTTTGATCTGGAAAGAGAGAATGCTTGTGCTACAAAAGAACAAAAGAATCACACCTTTCTAGGTTAAA
TCGCAACAAGTACATGGTGACAGATGGAGCAGCTTATATTGGAAATTTGATTGGGTAGGGAATGATTTCACTCAGAATG
CTGGCACGGGCCTTGTTATCAACCAGGCAGATGTGAGGAACAACAGAAGCATCATTAAAGCAACTTAAAGATGTGTTTGAA
AGGGACTGGTATTCACCGTATGCCAAAACCTTACAGCCAACCAACAGCCGAAGTCTCAAGCCTGTTCAAACCTCAAACC
CCTCTCCAACAAAACCTGCCACAGACGACACAGGCGGAAAGGATCCCCGGAACGTATGA

> Human PLD5 protein (445 amino acids) (SEQ ID NO: 128)

MGEDDGLSEKNCQNKCRIALVENIPEGLNYSNAPFHLSLFQGWMNLLNMAKKSVDIVSSHWDLNHHP SACQGQRLFE
 KLLQLTSQNI EIKLVSDVTADSKVLEALKLKGAEVTYMNMTAYNKGRLQSSFWIVDKQHVIYIGSAGLDWQSLGQMKE LGV
 IFYNC SCLVLDLQRIFALYSSLKFKSRVPQTWSKRLYGVYDNEKKLQLQLNETKSQAFVSN SPKLF C PKNRSFDIDAIYS
 VIDDAKQYVYIAVMDYLPISSTSTKRTYWPDLDAKIREALVLR SVRVRLLSFWKETDPLTFNF ISSLKAICTEIANCSL
 KVKFFDLERENACATKEQKNHTFPRLNRNKYMTDGAAYIGNFDWVGNDFTQNAGTGLVINQADVRNNRSI IKQLKDVFE
 RDWYSPYAKTLQPTKQPNCSSLFKLKPLSNKTATDDTGGKDPRNV

> Human PLD4-Ig fusion protein cDNA (2142 bp) (SEQ ID NO: 125)

ATGGAGTTTCAGACCCAGGTCTTTGTATTCTGTGTTGCTCTGGTTGTCTGGTGTGATGGAGattacaaggatgacgacga
 taaaGGATCCcccagaggggcccacaatcaagccctgtccctccatgcaaatgcccagcaccataacctcttggggtggaccat
 ccgtcttcatcttccctccaaagatcaaggatgtactcatgatctccctgagcccatagtcacatgtgtgggtggat
 gtgagcgaggatgaccagatgtccagatcagctggtttgtgaacaacgtggaagtacacacagctcagacacaaaccca
 tagagaggattacaacagtactctccgggtggctcagtgccctcccatccagcaccaggactggatgagtggaaggagt
 tcaaatgcaagggtcaacaacaagacctcccagcggccatcgagagaaccatctcaaaacccaaagggtcagtaagagct
 ccacaggtatatgtcttgccctccaccagaagaagagatgactaagaaacaggctactctgacctgcatggtcacagactt
 catgctgaagacatttacgtggagtggaaccaacaacgggaaaacagagctaaactacaagaacactgaaccagtcctgg
 actctgatggttcttacttcatgtacagcaagctgagagtggaagaagaactgggtggaaagaaatagctactcctgt
 tcagtggtccacgagggtctgcacaatcaccacacgactaagagcttctcccgactccgggtaaaCGTCCTCCACCTG
 GGGCCAGGTGCAGCCCAAGGACGTGCCAGGTCTGGGAGCATGGCTCCAGCCAGCTTGGGAGCCCCTGGAAGCAGAGG
 CCAGGCAGCAGAGGGACTCCTGCCAGCTTGTCTTGTGGAAAGCATCCCCAGGACCTGCCATCTGCAGCCGGCAGCCCC
 TCTGCCCAGCCTCTGGGCCAGGCCTGGCTGCAGCTGCTGGACACTGCCCAGGAGAGCGTCCACGTGGCTTCATACTACTG
 GTCCCTCACAGGGCCTGACATCGGGGTCAACGACTCGTCTTCCAGCTGGGAGAGGCTCTTCTGCAGAAGCTGCAGCAGC
 TGCTGGGCAGGAACATTTCCCTGGCTGTGGCCACCAGCAGCCCGACACTGGCCAGGACATCCACCGACCTGCAGGTTCTG
 GCTGCCCCGAGGTGCCCATGTACGACAGGTGCCCATGGGGCGGCTCACCAGGGGTGTTTTGCACTCCAAATTCTGGGTGT
 GGATGGACGGCACATATACATGGGCAGTGCCAAACATGGACTGGCGGTCTCTGACCGAGGTGAAGGAGCTTGGCGCTGTCA
 TCTATAACTGCAGCCACCTGGCCCAAGACCTGGAGAAGACCTTCCAGACCTACTGGGTACTGGGGGTGCCCAAGGCTGTC
 CTCCCCAAAACCTGGCCTCAGAACTTCTCATCTCACTTCAACCGTTTCCAGCCCTTCCACGGCCTCTTTGATGGGGTGCC
 CACCACTGCCTACTTCTCAGCGTCGCCACCAGCACTCTGTCCCCAGGGCCGACCCGGGACCTGGAGGCGCTGCTGGCGG
 TGATGGGGAGCGCCCAGGAGTTCATCTATGCCTCCGTGATGGAGTATTTCCCCACCACGCGCTTCAGCCACCCCCGAGG
 TACTGGCCGGTGCTGGACAACGCGCTGCGGGCGGCAGCCTTCGGCAAGGGCGTGCGCGTGCGCCTGCTGGTGGCTGCGG
 ACTCAACACGGACCCACCATGTTCCCTACCTGCGGTCCCTGCAGGCGCTCAGCAACCCCGCGGCCAACGTCTCTGTGG
 ACGTGAAAGTCTTCATCGTGCCGGTGGGGAACCATTTCCAACATCCATTTCAGCAGGGTGAACCACAGCAAGTTCATGGTC
 ACGGAGAAGGCAGCCTACATAGGCACCTCCAACCTGGTCGGAGGATTACTTCAGCAGCAAGGCGGGGGTGGGCTTGGTGGT
 CACCCAGAGCCCTGGCGCGCAGCCCGCGGGGGCCACGGTGCAGGAGCAGCTGCGGCAGCTCTTTGAGCGGGACTGGAGTT
 CGCGCTACGCCGTCCGGCTGGACGGACAGGCTCCGGGCCAGGACTGCGTTTGGCAGGGCTGA

> Human PLD4-Ig fusion protein (713 amino acids) (SEQ ID NO: 126)

MEFQTQVFVFLWLSGVDGDYKDDDDKGSPPRGPTIKPCPPCKCPAPNLLGGPSVFIFFPKIKDVLMI SLSP IVTCVVVD
VSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQQWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRA
PQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSC
SVVHEGLHNHHTTKSFSRTPGKRPTWGQVQPKDVPRSWEHGSSPAWEPLAEARQQRDSCQLVLVESIPQDLPSAAGSP
SAQPLGQAWLQLLDTAQESVHVASYWSLTGPDIGVNDSSSQLGEALLQKLQQLGRNISLAVATSSPTLARTSTD LQVL
AARGAHVRQVPMGRLTRGVLHSEFWVVDGRHIYMGSANMDWRSLTQVKELGAVIYNCSHLAQDLEKTFQTYWVLGVPKAV
LPKTWPQNFSSHFNRFPFHGLFDGVPTTAYFSASPPALCPQGRTRDLEALLAVMGSAQEFIYASVMEYFPTTRFSHP
YWPVLDNALRAAAFSGKGVRRLLVGCGLNTDPTMFPYLRSLQALSNPAAVSVVDVKVFI VPGVGNHSNIPFSRVNHSKFMV
TEKAAYIGTSNWSYDFSSSTAGVGLVVTQSPGAQAPAGATVQEQLRQLFERDWSSRYAVGLDGQAPGQDCVWQG

[Accession Number]

[0144]

NITE ABP-1211

NITE ABP-1212

5 NITE ABP-1213

NITE ABP-1214

[Sequence list free text]

[0145]

SEQ ID NO 45: Forward primer

10 SEQ ID NO 46: Reverse primer

SEQ ID NO 47: Forward primer

SEQ ID NO 48: Reverse primer

SEQ ID NO 49: Forward primer

SEQ ID NO 50: Reverse primer

15 SEQ ID NO 51: Forward primer

SEQ ID NO 52: Reverse primer

SEQ ID NO 53: Forward primer

SEQ ID NO 54: Reverse primer

SEQ ID NO 70: Anchor primer

20 SEQ ID NO 70: n is deoxyinosine

SEQ ID NO 71: AUAP primer

SEQ ID NO 72: Primer

SEQ ID NO 73: Primer

SEQ ID NO 114: Primer

25 SEQ ID NO 115: Primer

SEQ ID NO 116: Primer

SEQ ID NO 117: Primer

WO 2013/115410

PCT/JP2013/052781

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SEQ ID NO 118: Primer

SEQ ID NO 119: Primer

CLAIMS

1. A monoclonal antibody that binds to a phospholipase D4 (PLD4) protein, or a fragment containing an antigen-binding region thereof.
2. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SYWMH as CDR1, the sequence DIYPGSDSTNYNEKFKS as CDR2 and the sequence GGWLDAMDY as CDR3 in the heavy chain variable region.
3. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence RASQDISNYLN as CDR1, the sequence YTSRLHS as CDR2 and the sequence QQGNTLPW as CDR3 in the light chain variable region.
4. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SYWMH as CDR1, the sequence DIYPGSDSTNYNEKFKS as CDR2 and the sequence GGWLDAMDY as CDR3 in the heavy chain variable region, and has the sequence RASQDISNYLN as CDR1, the sequence YTSRLHS as CDR2, and the sequence QQGNTLPW as CDR3 in the light chain variable region.
5. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence TYWMH as CDR1, the sequence AIYPGNSETSYNQKFKG as CDR2, and the sequence GYSDFDY as CDR3 in the heavy chain variable region.
6. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence HASQGIRSNIG as CDR1, the sequence HGTNLED as CDR2, and the sequence VQYVQFP as CDR3 in the light chain variable region.
7. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence TYWMH as CDR1, the sequence AIYPGNSETSYNQKFKG as CDR2, and the sequence GYSDFDY as CDR3 in the heavy chain variable region, and has the sequence HASQGIRSNIG as CDR1, the sequence HGTNLED as CDR2, and the sequence VQYVQFP as CDR3 in the light chain variable region.
8. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence DYNLH as CDR1, the sequence YIYPYNGNTGYNQKFKR as CDR2, and the sequence GGIYDDYYDYAIDY as CDR3 in the heavy chain variable region.
9. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence RASENIYSHIA as CDR1, the sequence GATNLAH as CDR2, and the sequence QHFWGTP as CDR3 in the light chain variable region.

10. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence DYNLH as CDR1, the sequence YIYPYNGNTGYNQKFKR as CDR2, and the sequence GGIYDDYDYDAIDY as CDR3 in the heavy chain variable region, and has the sequence RASENIYSHIA as CDR1, the sequence GATNLAH as CDR2, and the sequence QHFWGTP as CDR3 in the light chain variable region.

11. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SYLY as CDR1, the sequence LINPTNSDTIFNEKFKS as CDR2, and the sequence EGGYGYGPFAY as CDR3 in the heavy chain variable region (8C11 antibody).

12. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence TSSQTLVHSNGNTYLH as CDR1, the sequence KVSNRFS as CDR2, and the sequence HSTHVP as CDR3 in the light chain variable region.

13. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SYLY as CDR1, the sequence LINPTNSDTIFNEKFKS as CDR2, and the sequence EGGYGYGPFAY as CDR3 in the heavy chain variable region, and has the sequence TSSQTLVHSNGNTYLH as CDR1, the sequence KVSNRFS as CDR2, and the sequence HSTHVP as CDR3 in the light chain variable region.

14. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SYGMS as CDR1, the sequence TISSGGSYIYPESVKG as CDR2, and the sequence LYGGRRGYGLDY as CDR3 in the heavy chain variable region.

15. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence RSSKSLHSDGITYLY as CDR1, the sequence QMSNLAS as CDR2, and the sequence AQNLEL as CDR3 in the light chain variable region.

16. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SYGMS as CDR1, the sequence TISSGGSYIYPESVKG as CDR2, and the sequence LYGGRRGYGLDY as CDR3 in the heavy chain variable region, and has the sequence RSSKSLHSDGITYLY as CDR1, the sequence QMSNLAS as CDR2, and the sequence AQNLEL as CDR3 in the light chain variable region.

17. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SHYYWT as CDR1, the sequence

YISYDGSNNYNPSLKN as CDR2, and the sequence EGPLYYGNPYWYFDV as CDR3 in the heavy chain variable region.

18. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2, and the sequence QQFNTLP as CDR3 in the light chain variable region.

19. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SHYYWT as CDR1, the sequence YISYDGSNNYNPSLKN as CDR2, and the sequence EGPLYYGNPYWYFDV as CDR3 in the heavy chain variable region, and has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2, and the sequence QQFNTLP as CDR3 in the light chain variable region.

20. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SHYYWS as CDR1, the sequence YISYDGSNNYNPSLKN as CDR2, and the sequence EGPLYYGNPYWYFDV as CDR3 in the heavy chain variable region.

21. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2, and the sequence QQFNTLP as CDR3 in the light chain variable region.

22. The monoclonal antibody, or a fragment containing an antigen-binding region thereof according to claim 1, which has the sequence SHYYWS as CDR1, the sequence YISYDGSNNYNPSLKN as CDR2, and the sequence EGPLYYGNPYWYFDV as CDR3 in the heavy chain variable region, and has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2, and the sequence QQFNTLP as CDR3 in the light chain variable region.

23. A monoclonal antibody, or a fragment containing an antigen-binding region thereof, being produced by any one of hybridomas mp5B7, mp7B4, mp13D4, and mp13H11 that are deposited under Accession Numbers: NITE ABP-1211, NITE ABP-1212, NITE ABP-1213, and NITE ABP-1214.

24. A hybridoma, producing any one of the monoclonal antibodies according to claim 1 or 2.

25. A hybridoma mp5B7, mp7B4, mp13D4 or mp13H11, being deposited under Accession Numbers: NITE ABP-1211, NITE ABP-1212, NITE ABP-1213 or NITE ABP-1214.

26. A method of preparing a monoclonal antibody, comprising: culturing the hybridoma according to claim 25; and collecting a monoclonal antibody from the culture.

27. A method of preparing a cell that produces a monoclonal antibody that binds to PLD4, comprising the following processes:

1) a process of administering recombinant PLD4-Ig fusion protein that encodes an amino acid sequence containing a PLD4 extracellular domain, to an immune animal; and

2) a process of selecting an antibody-producing cell that produces an antibody that binds to PLD4 from antibody-producing cells of the immune animal.

28. The method according to claim 27, wherein the cell that expresses PLD4 is a cell that retains an extrinsic polynucleotide that encodes an amino acid sequence containing the PLD4 extracellular domain in an expressible way.

29. The method according to claim 28, wherein the cell is an animal cell.

30. The method according to claim 29, wherein the cell is a human-derived cell.

31. The method according to claim 30, wherein the human-derived cell is a HEK-293T cell.

32. The method according to any one of claims 27 to 31, comprising a process of cloning the obtained antibody-producing cells incrementally.

33. A method of preparing a monoclonal antibody that binds to a PLD4 extracellular domain, comprising: culturing the antibody-producing cells obtained by the method according to claim 29; and collecting a monoclonal antibody from the culture.

34. A monoclonal antibody that recognizes PLD4, or a fragment containing an antigen-binding region thereof, which can be obtained by the following processes;

1) administering a recombinant PLD4-Ig fusion protein that encodes an amino acid sequence containing a PLD4 extracellular domain, to an immune animal,

2) selecting an antibody-producing cell that produces an antibody that binds to PLD4 from antibody-producing cells of the immune animal, and

3) culturing the antibody-producing cells selected in the process 2), and collecting an antibody that recognizes PLD4 from the culture.

35. An immunogen for preparing an antibody that binds to PLD4, comprising (a) an animal cell that retains a polynucleotide that encodes an amino acid sequence containing a PLD4 extracellular domain extrinsically in an expressible way, or a cell membrane fraction thereof.

36. The immunogen according to claim 35, wherein the animal cell is a human-derived cell.

37. A method of detecting a plasmacytoid dendritic cell, comprising: bringing a monoclonal antibody that binds to a PLD4 extracellular domain, or a fragment containing an antigen-binding region thereof into contact with a test cell; and detecting a monoclonal antibody that binds to the cell, or a fragment containing an antigen-binding region thereof.

38. A reagent for detection of a plasmacytoid dendritic cell, comprising: a

monoclonal antibody that binds to a PLD4 extracellular domain; or a fragment containing an antigen-binding region thereof.

39. A method of suppressing an activity of a plasmacytoid dendritic cell, comprising: bring any one of the components described below into contact with the plasmacytoid dendritic cell,

(a) a monoclonal antibody that binds to PLD4 and suppresses an activity of the plasmacytoid dendritic cell, or a fragment containing an antigen-binding region thereof, and

(b) an immunoglobulin in which a complementarity-determining region of the monoclonal antibody (a) is transplanted, or a fragment containing an antigen-binding region thereof.

40. A method of suppressing an activity of a plasmacytoid dendritic cell in a living organism, comprising: administering any one of the components described below to the living organism,

(a) a monoclonal antibody that binds to PLD4, and suppresses an activity of the plasmacytoid dendritic cell, or a fragment containing an antigen-binding region thereof, and

(b) an immunoglobulin in which a complementarity-determining region of the monoclonal antibody (a) is transplanted, or a fragment containing an antigen-binding region thereof.

41. The method according to claim 39 or 40, wherein the activity of a plasmacytoid dendritic cell is either one of interferon producing activity and survival of an interferon producing cell, or both of them.

42. An agent for suppressing an activity of a plasmacytoid dendritic cell, comprising: any one of the components described below as an active component,

(a) a monoclonal antibody that binds to PLD4, and suppresses an activity of the plasmacytoid dendritic cell, or a fragment containing an antigen-binding region thereof, and

(b) an immunoglobulin in which a complementarity-determining region of the monoclonal antibody (a) is transplanted, or a fragment containing an antigen-binding region thereof.

43. The agent for suppressing an activity of an interferon producing cell according to claim 42, wherein the activity of a plasmacytoid dendritic cell is either one of interferon producing activity and survival of an interferon producing cell, or both of them.

FIG. 1

Transmembrane domain										Outer 2PLD domain (HKD motif)									
10	20	30	40	50	60														
MLKPLWKA	AV APTWPCSMPP	RRPWDREAGT	LQVLGALAVL	WLGSVALICL	LWQVPRPPTW														
70	80	90	100	110	120														
GQVQPKDVPR	SWEHGSSPAW	EPLEAEARQQ	RDSCQLVLVE	SIPQDLPSAA	GSPSAQPLGQ														
130	140	150	160	170	180														
AWLQLLDTAQ	ESVHVASYW	SLTGPDIGVN	DSSSQLGEAL	LQKLQQLLGR	NISLAVATSS														
190	200	210	220	230	240														
PTLARTSTD	L QVLAARGAHV	RQVPMGRLTR	GVLHSKFWVV	DGRHIYMGSA	NMDWRSLTQV														
250	260	270	280	290	300														
KELGAVIYNC	SHLAQDLEKT	FQTYWVLGVP	KAVLPKTWPQ	NFSSHFNRFQ	PFHGLFDGVP														
310	320	330	340	350	360														
TTAYFSASPP	ALCPQGRTRD	LEALLAVMGS	AQEFIYASVM	EYFPTTRFSH	PPRYWPVLDN														
370	380	390	400	410	420														
ALRAAAF	GKGVRRVRLLVGCG	LNTDPTMFPY	LRSLQALSNP	AANVSVDVKV	FIVPVGNHSN														
430	440	450	460	470	480														
IPFSRVNHSK	FMVTEKAAYI	GTSNWS	EDYF SSTAGVGLV	TQSPGAQPAG	ATVQEQLRQL														
490	500																		
FERDWSSRYA	VGLDGQAPGQ	DCVWQ																	

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FIG. 2

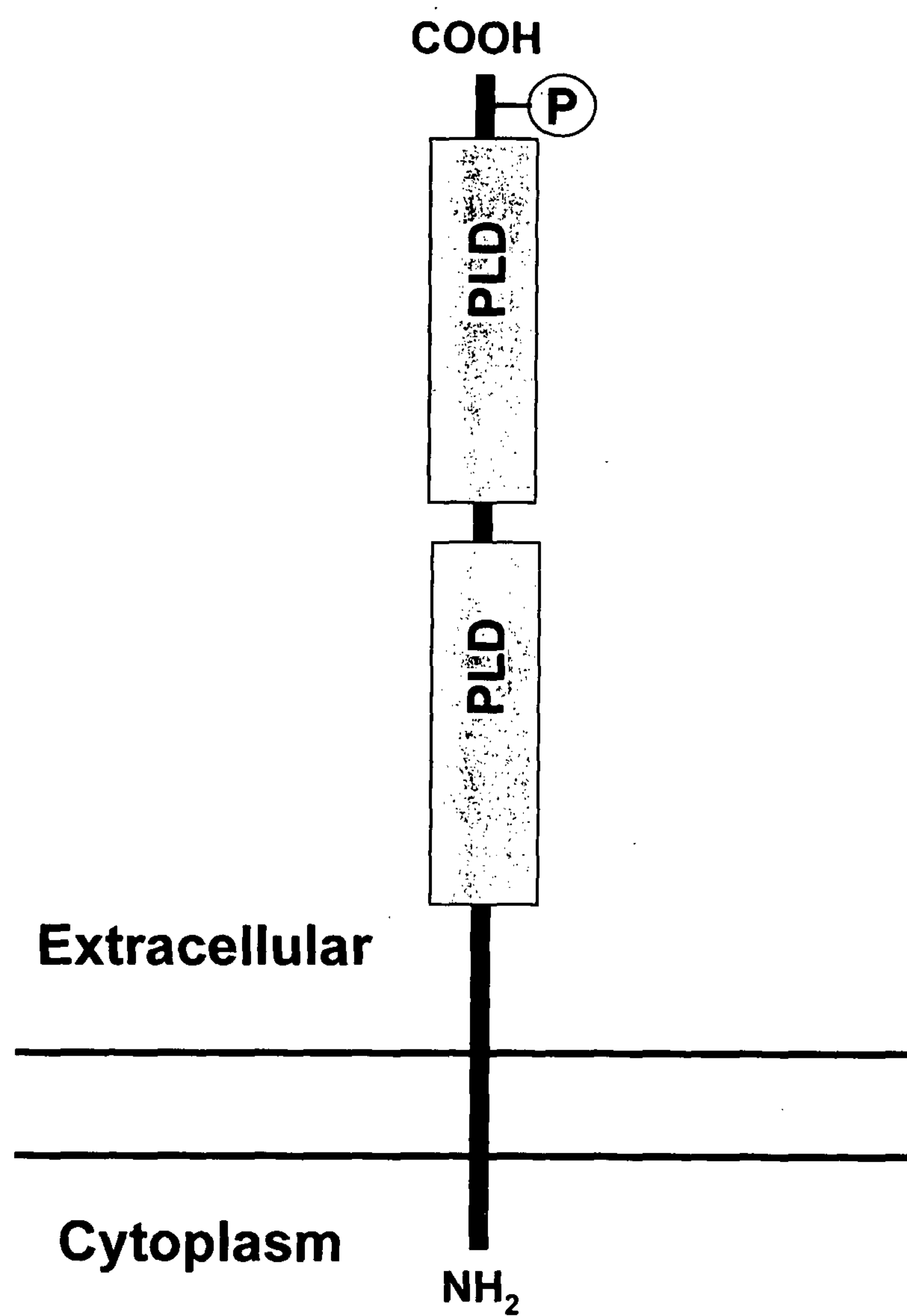
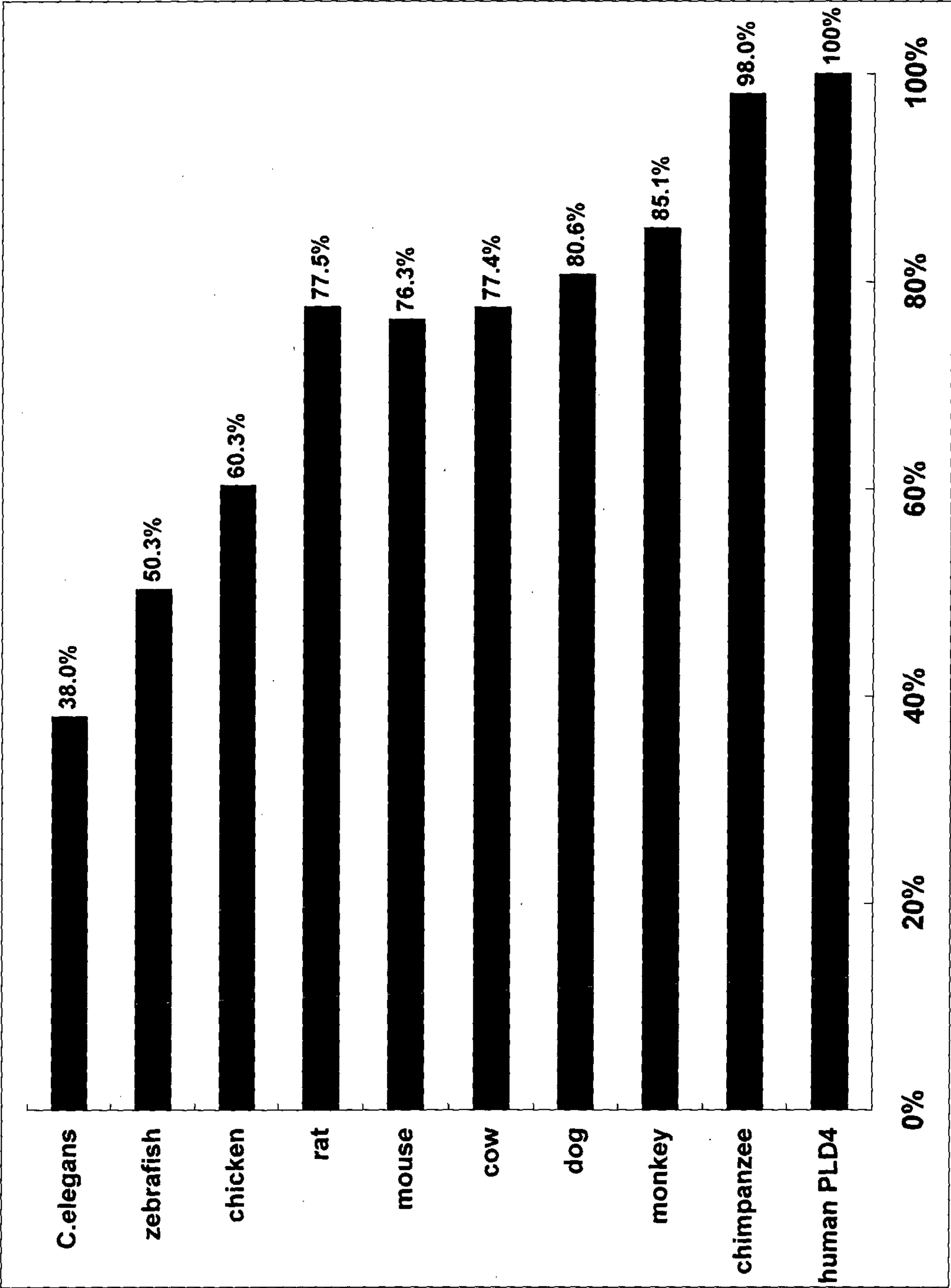
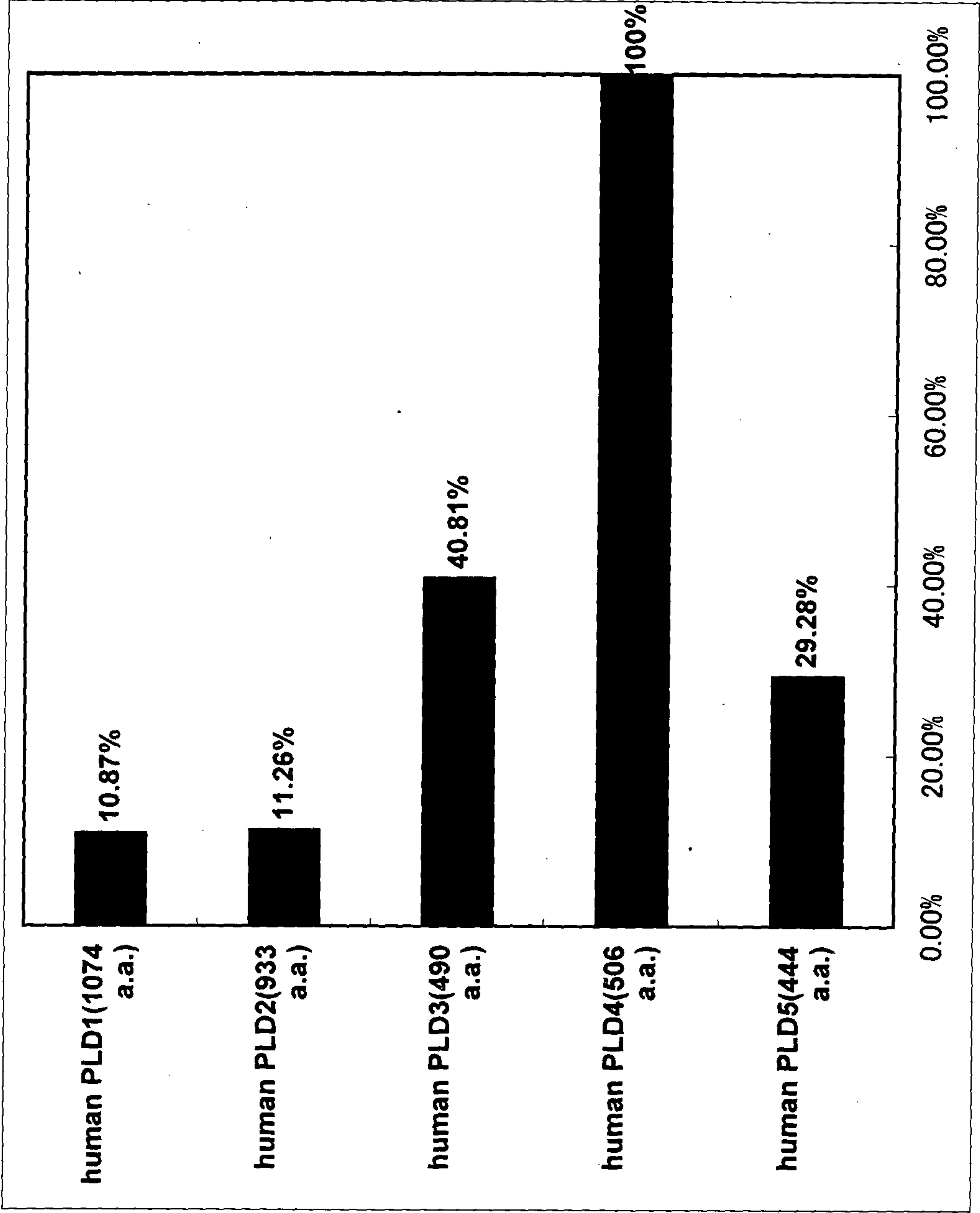


FIG. 3



Identity of protein sequences

FIG. 4



Identity of protein sequences

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FIG. 5

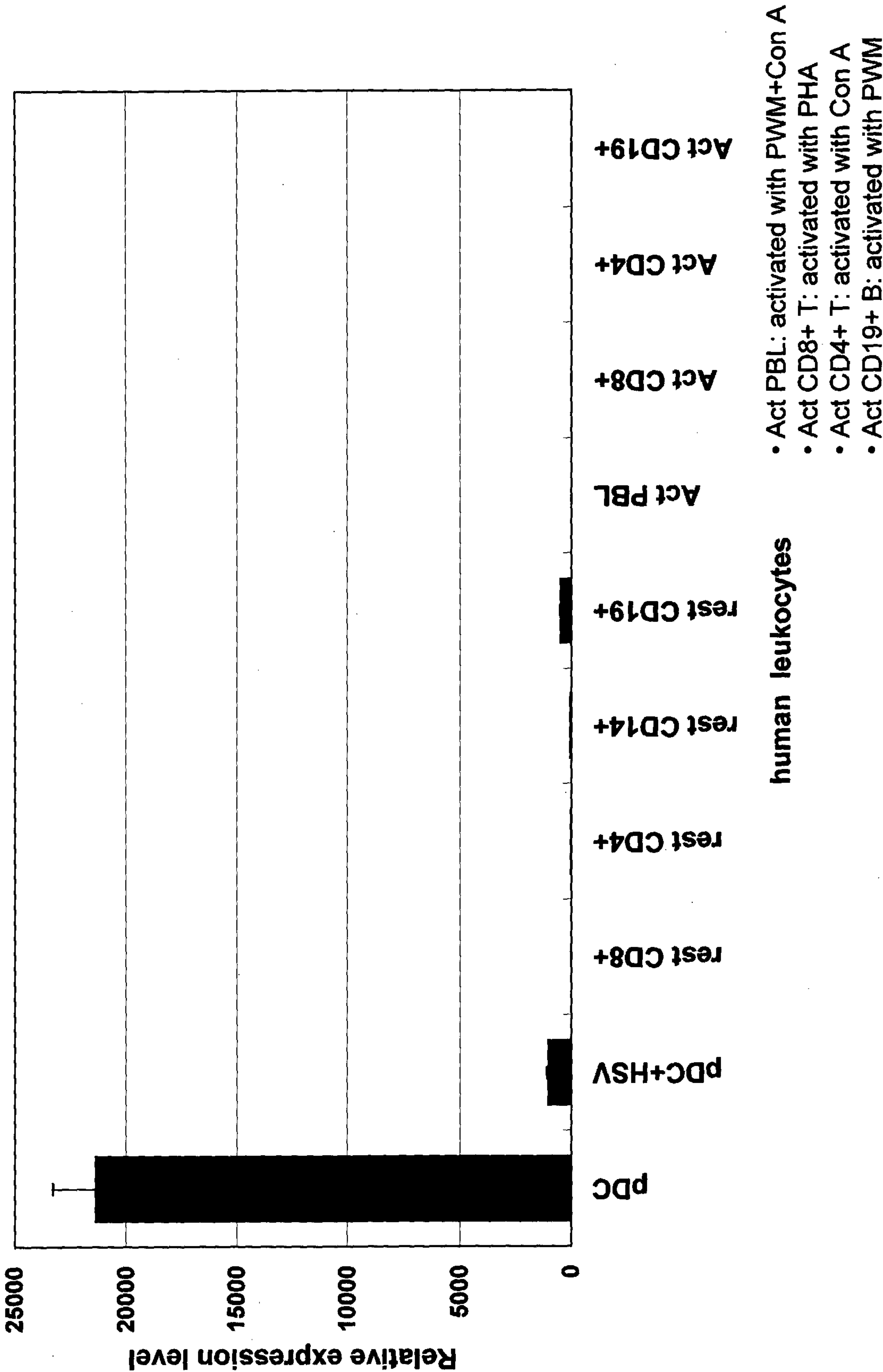


FIG. 6

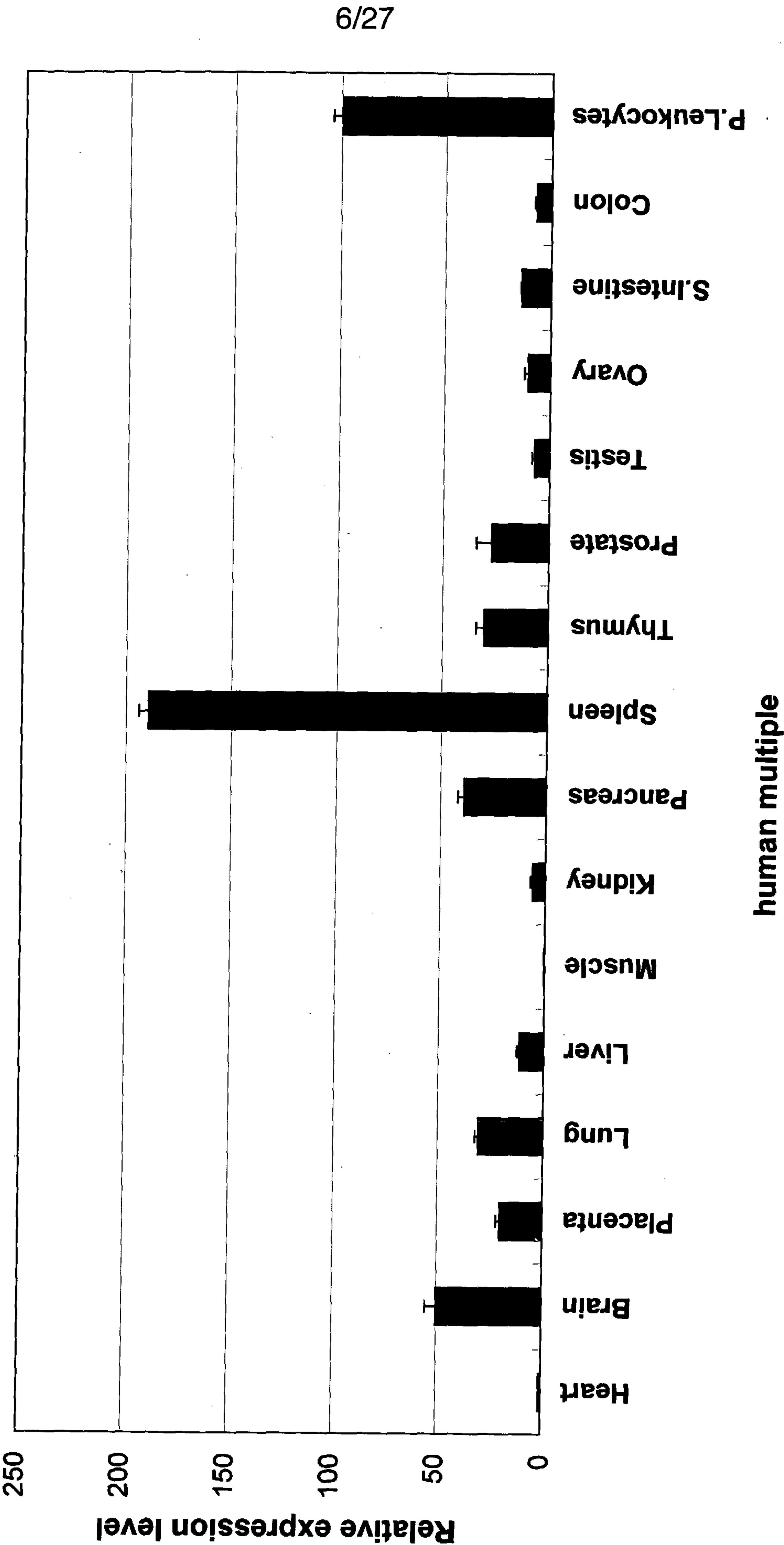
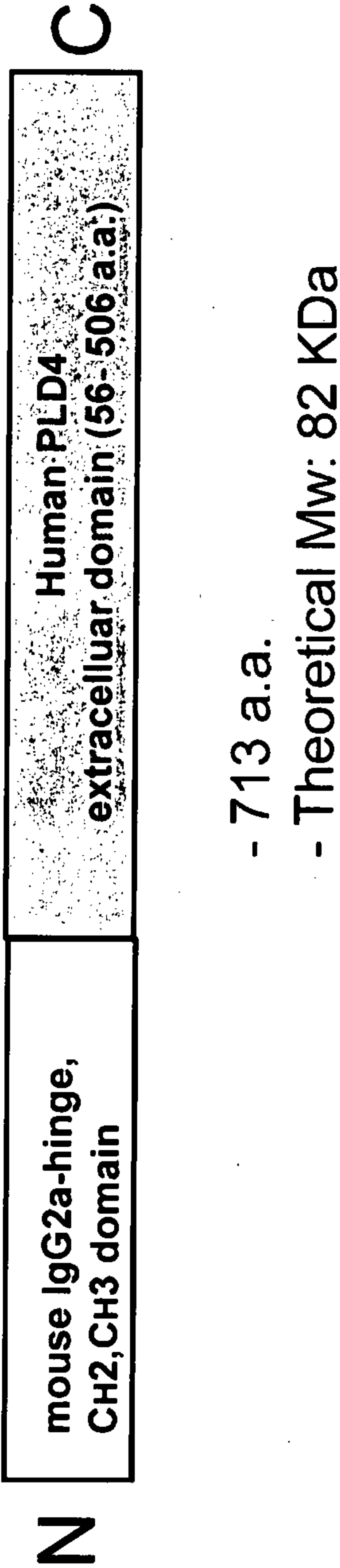
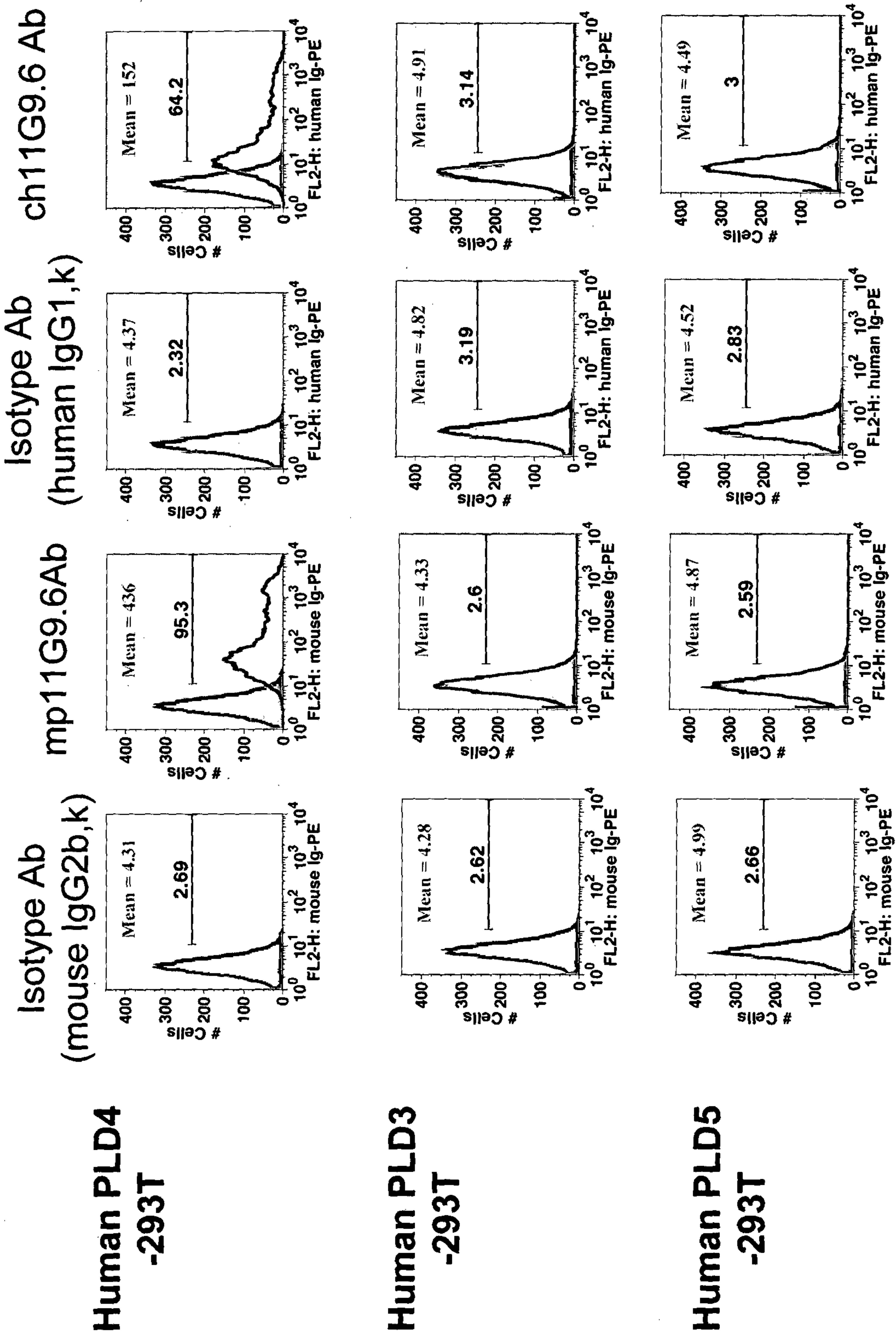


FIG. 7



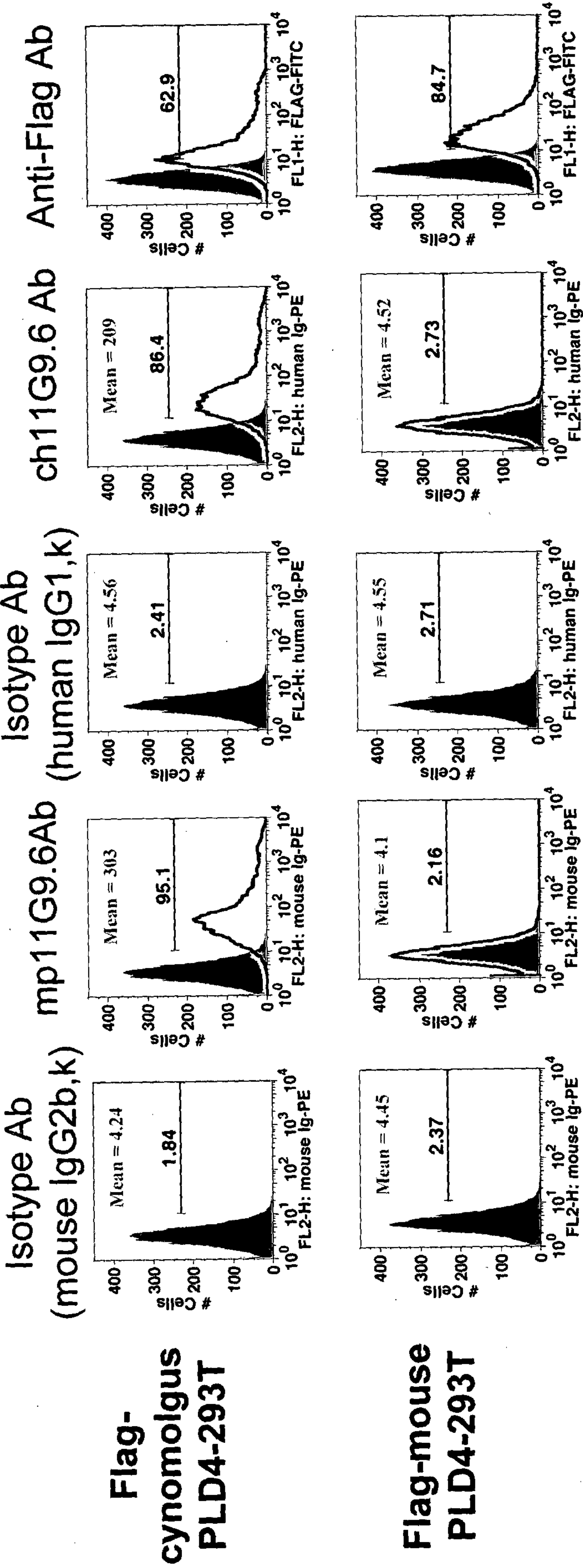
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FIG. 8



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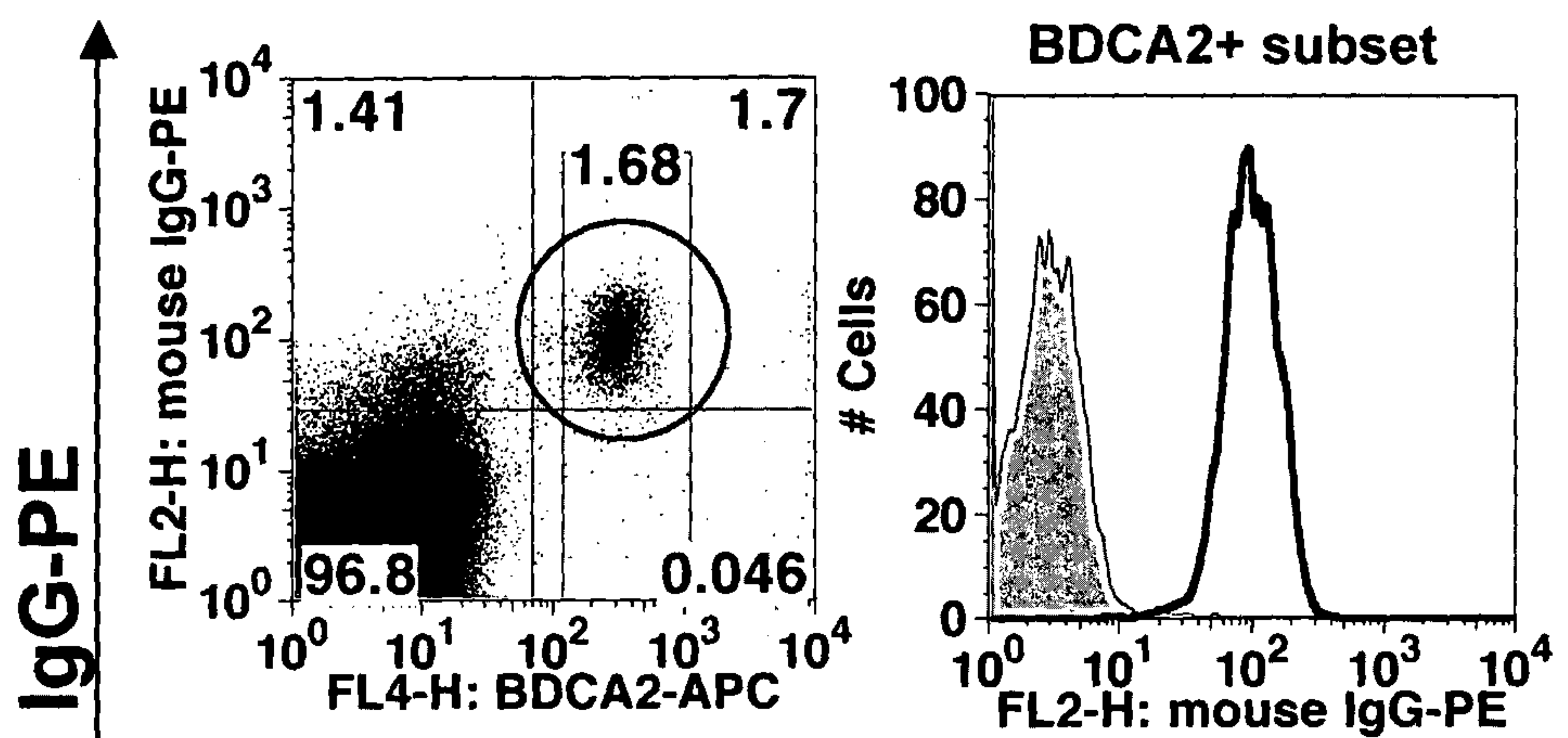
FIG. 9



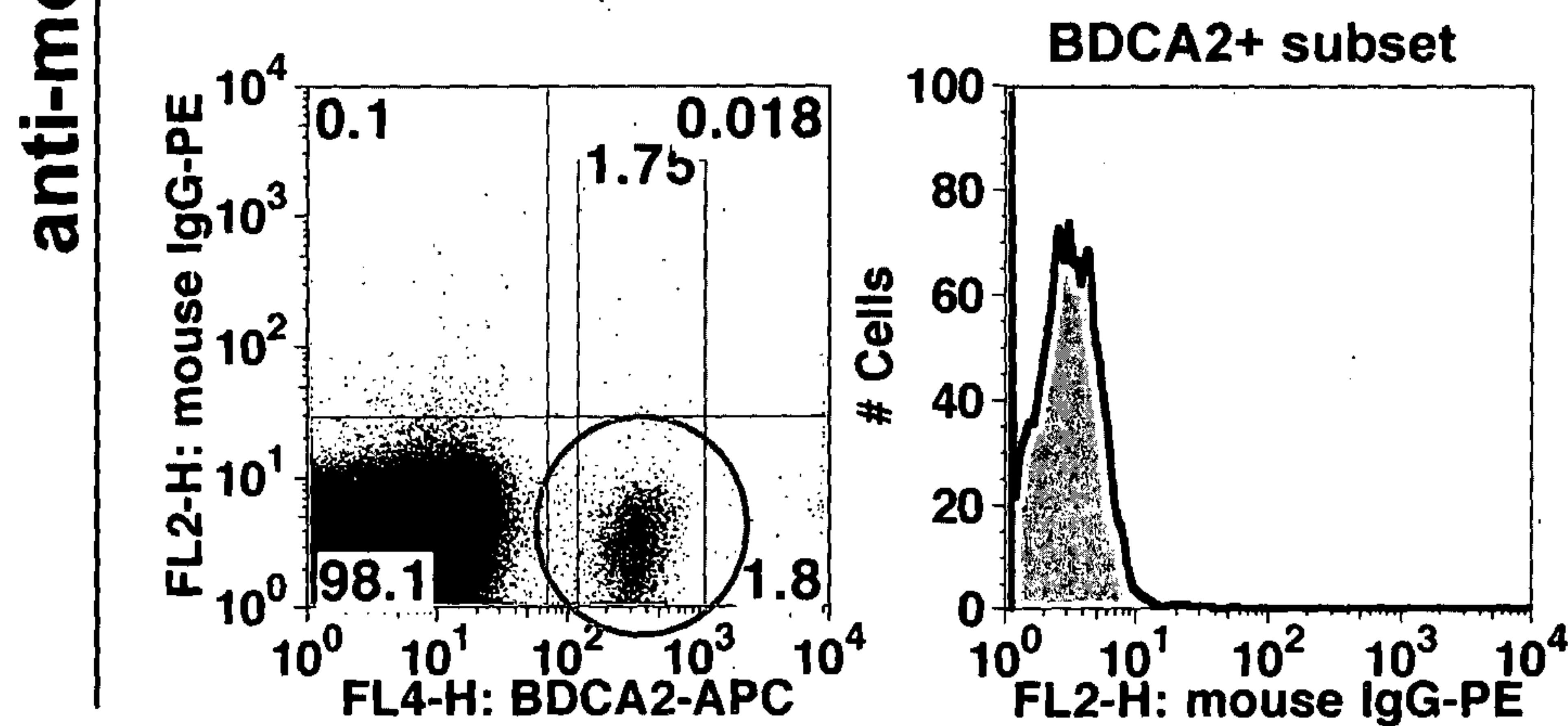
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FIG. 10

mp11G9.6 Ab



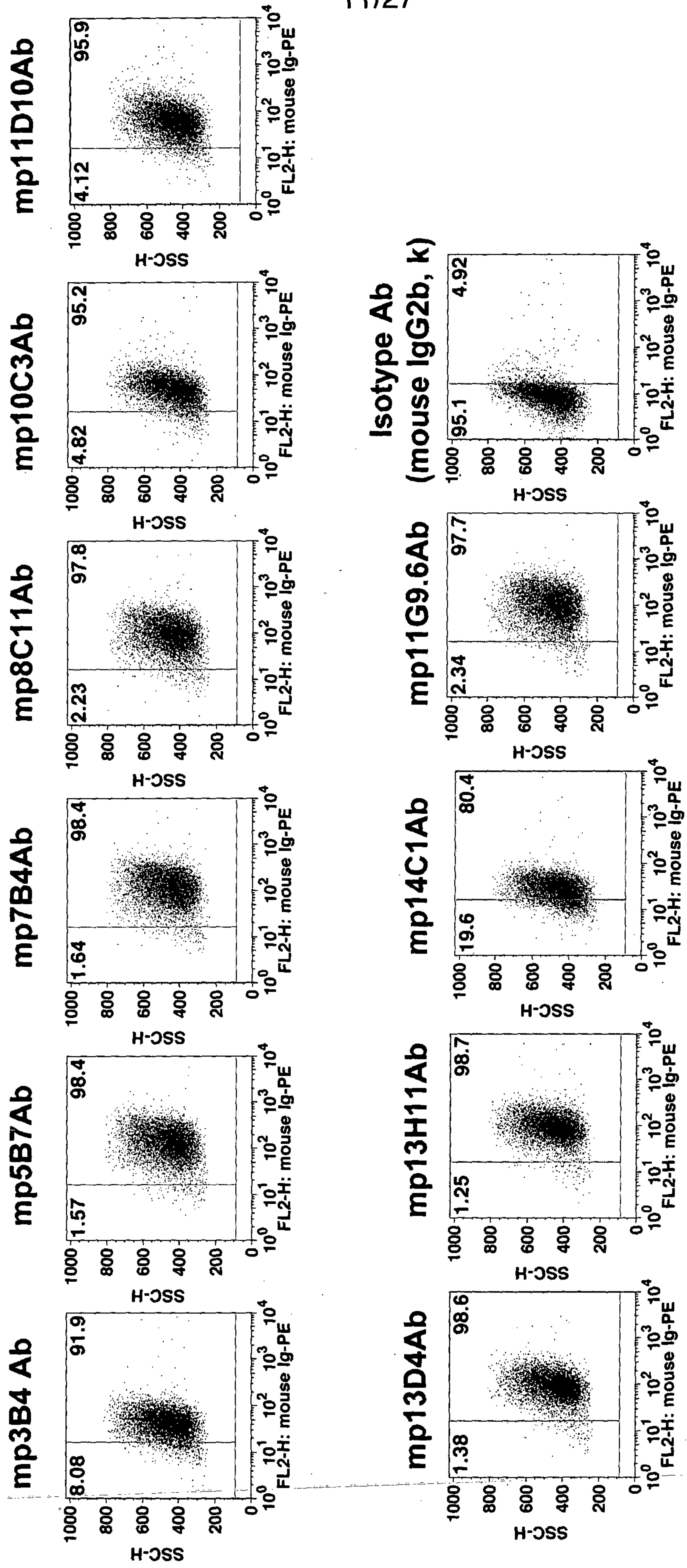
Isotype Ab (mouse IgG2b, k)



BDCA2

(Ab conc.: 10ug/ml)

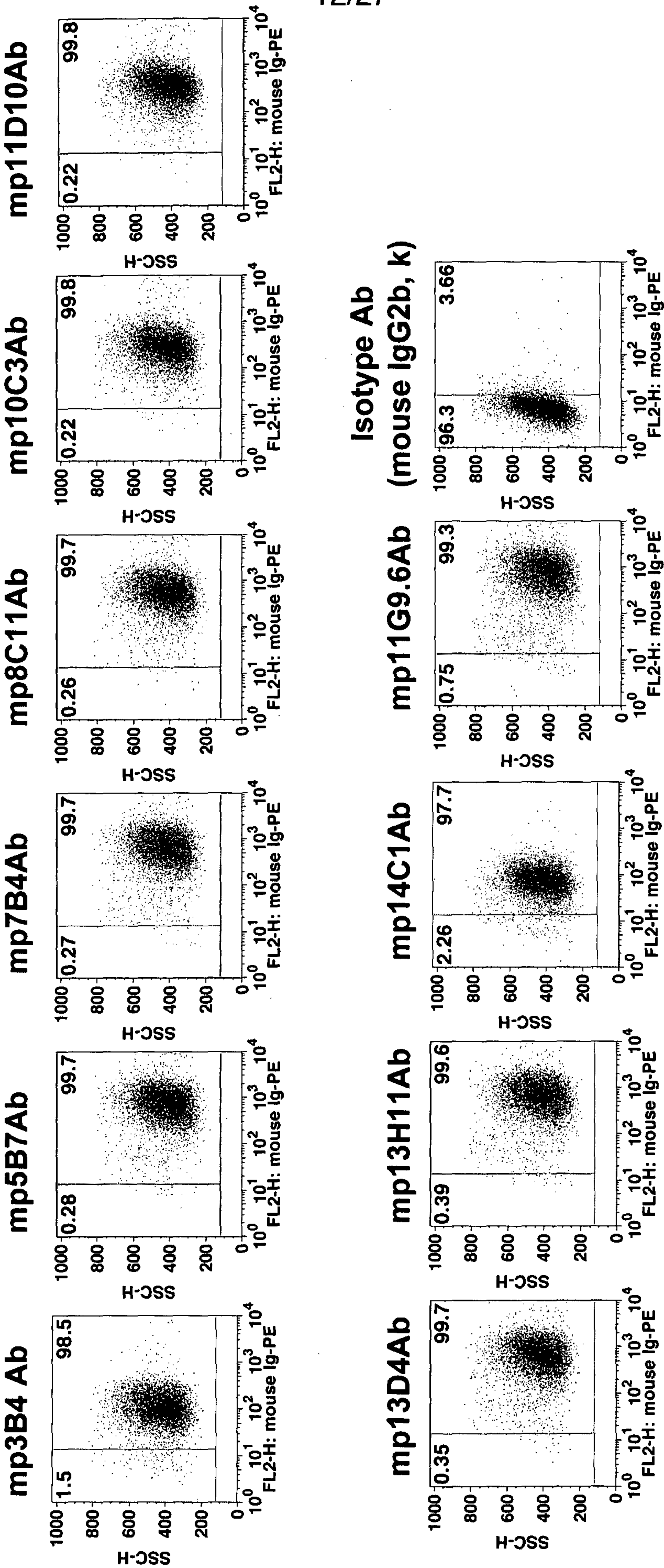
FIG. 11



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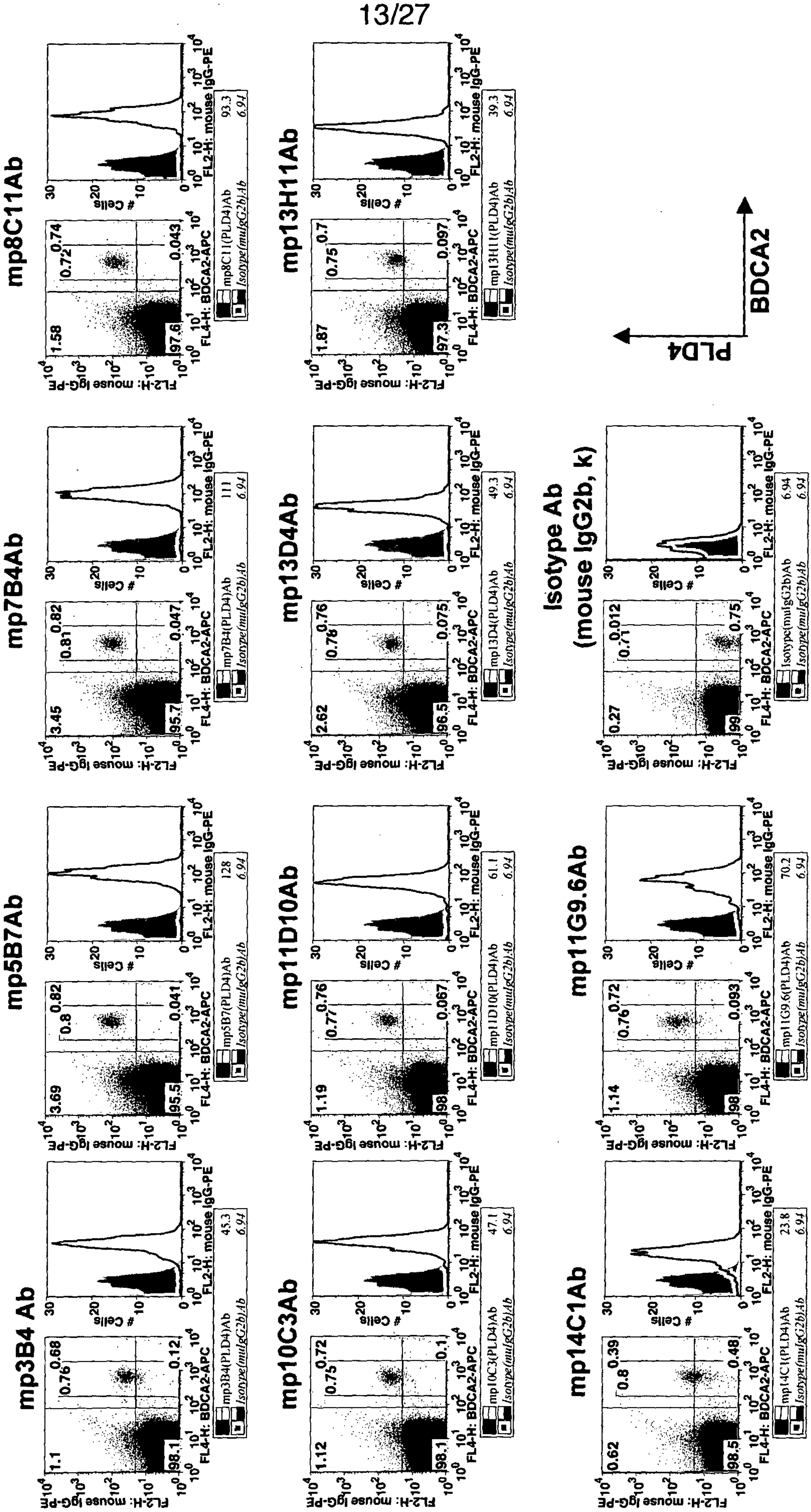
(Ab conc.: 5 ug/ml)

FIG. 12



(Ab conc.: 5 ug/ml)

FIG. 13



(Ab conc.: 10 ug/ml)

- Human PLD4: 506 a.a.
- Cynomolgus PLD4: 506 a.a. (94.4 % homology)
- Rhesus PLD4: 506 a.a. (93.9 % homology)
- Mouse PLD4: 503 a.a. (72.5 % homology)

FIG. 15

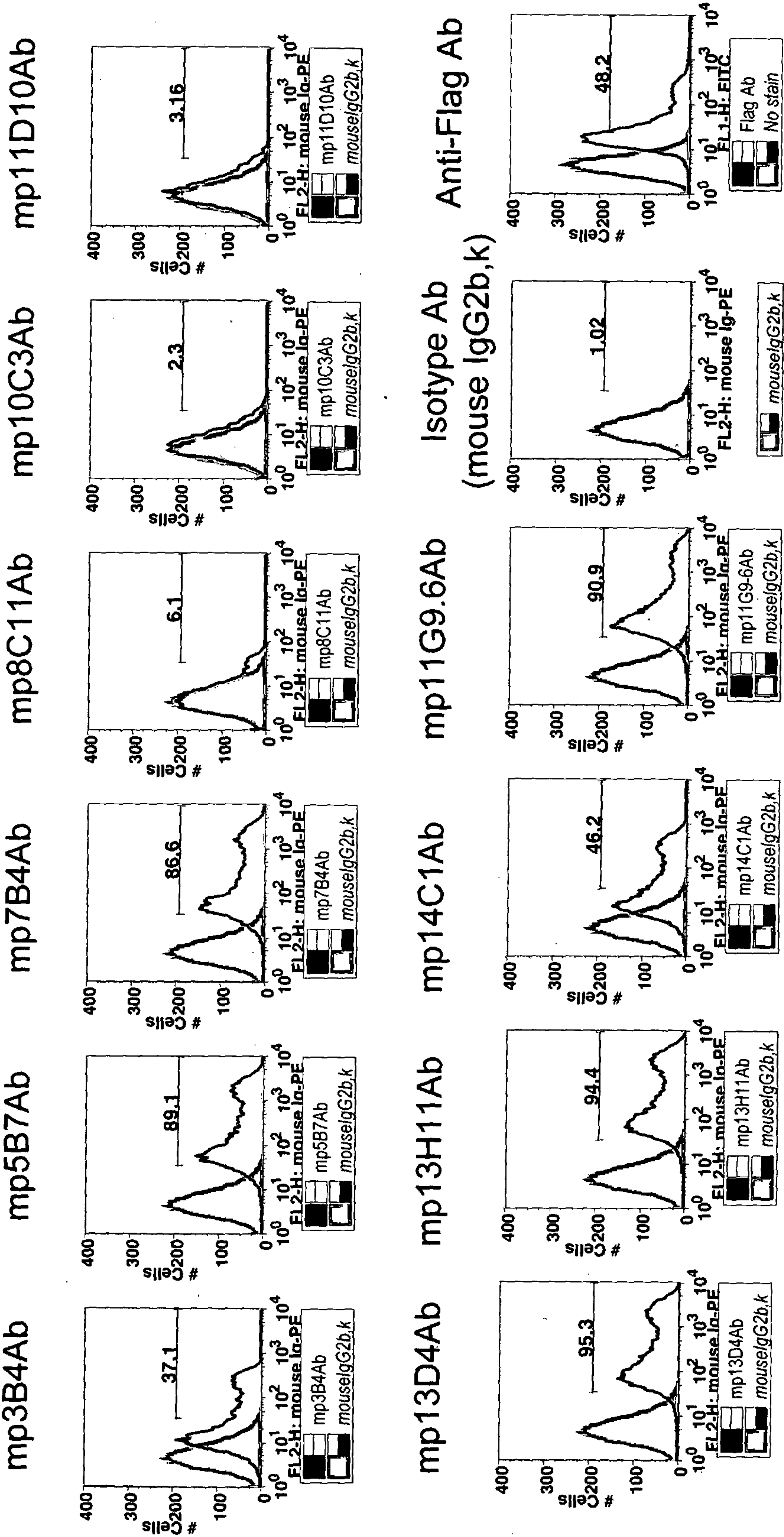
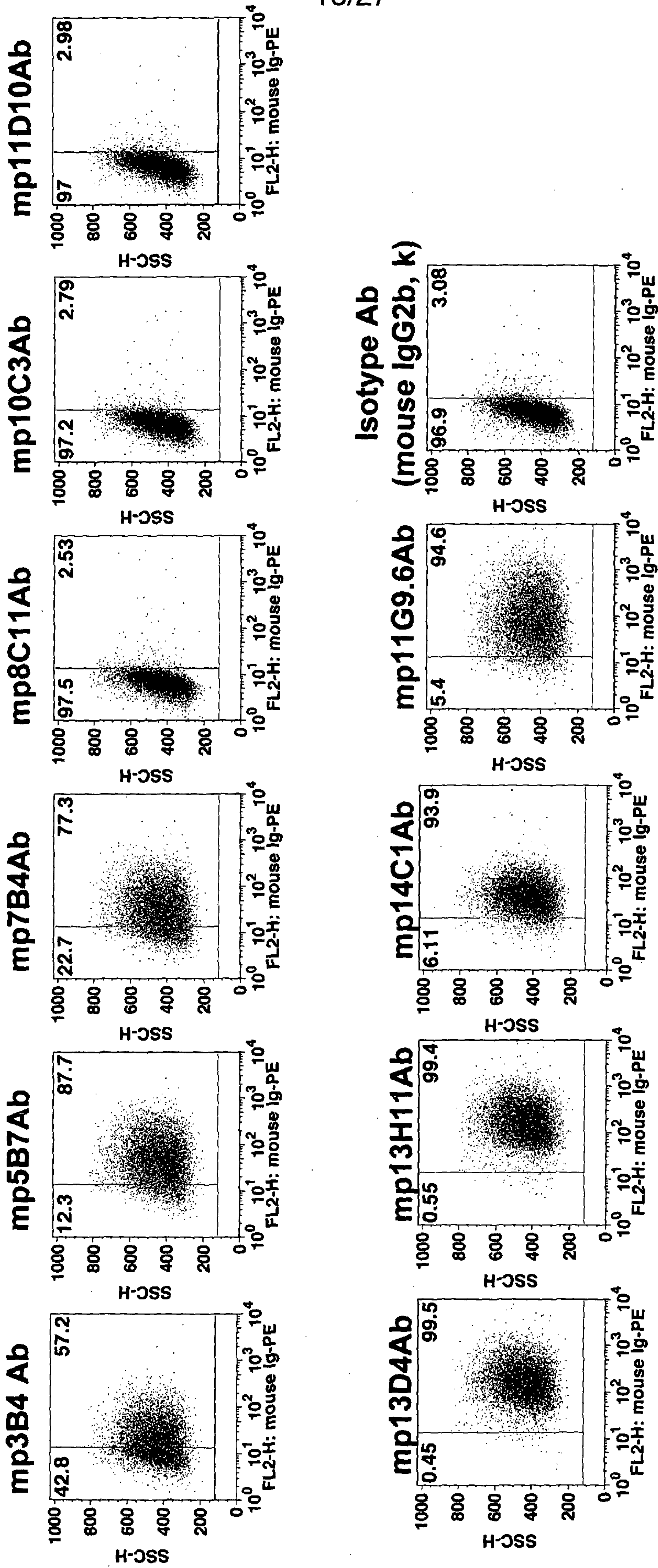


FIG. 16



(Ab conc.: 5 ug/ml)

FIG. 17

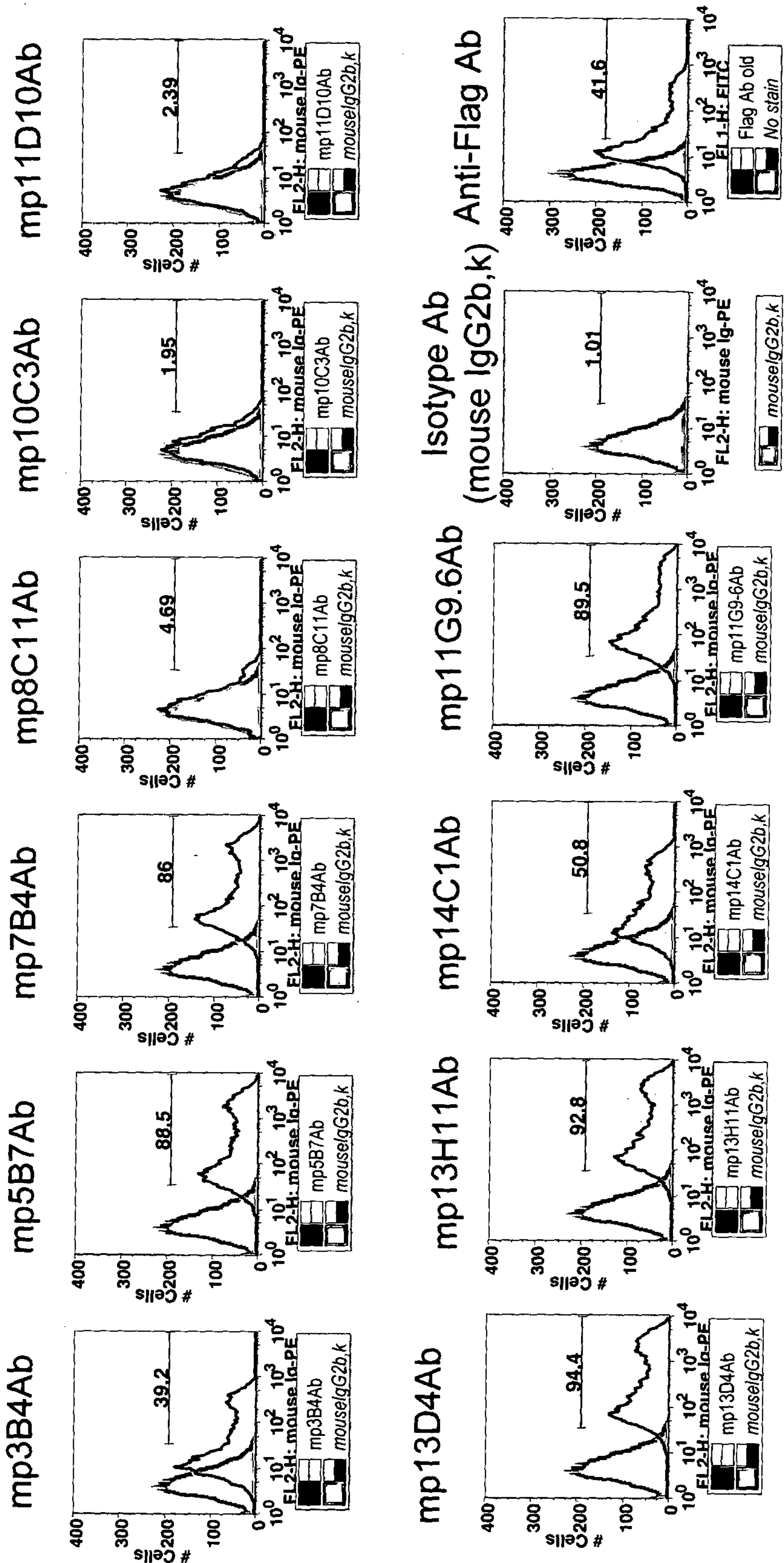


FIG. 18-1

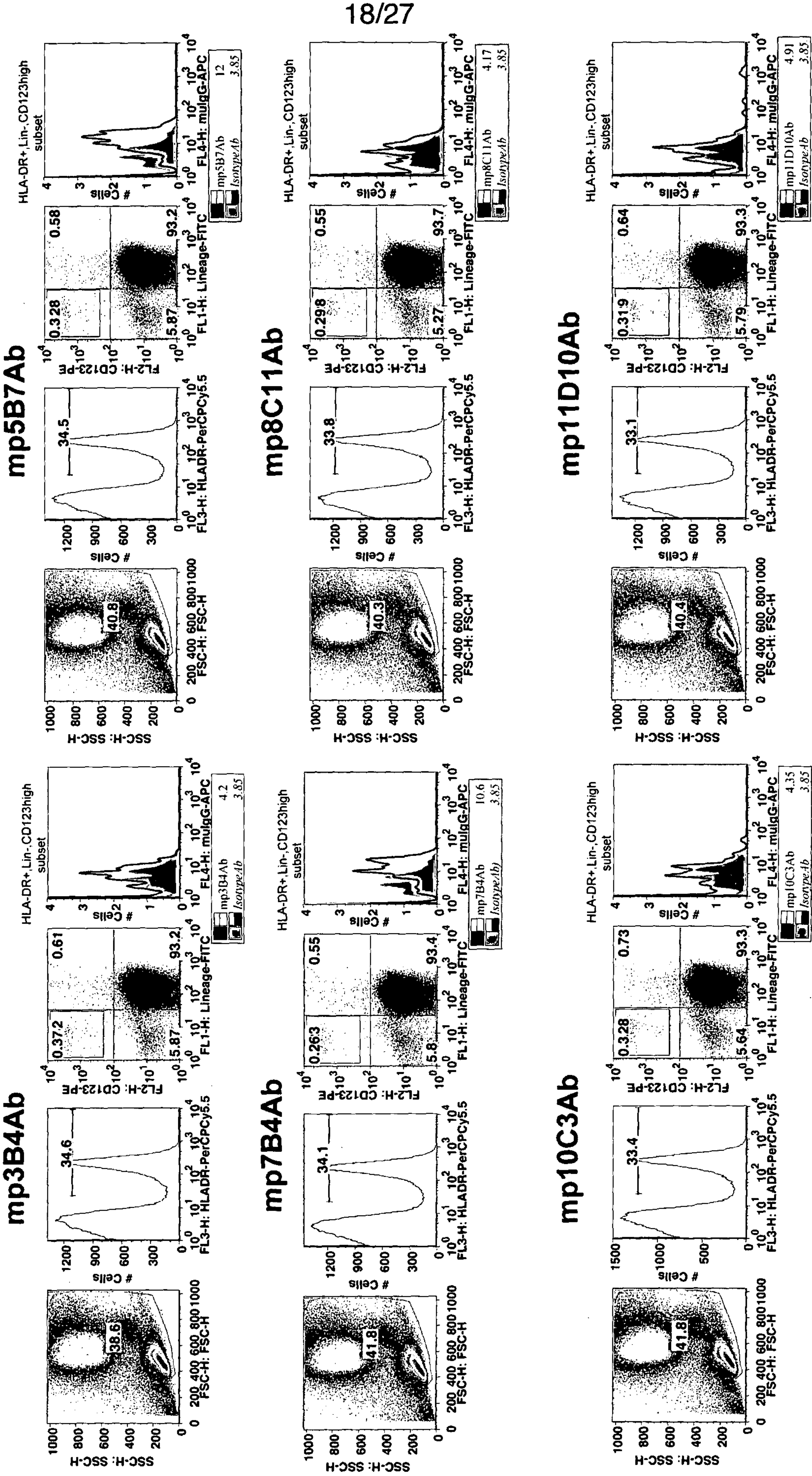
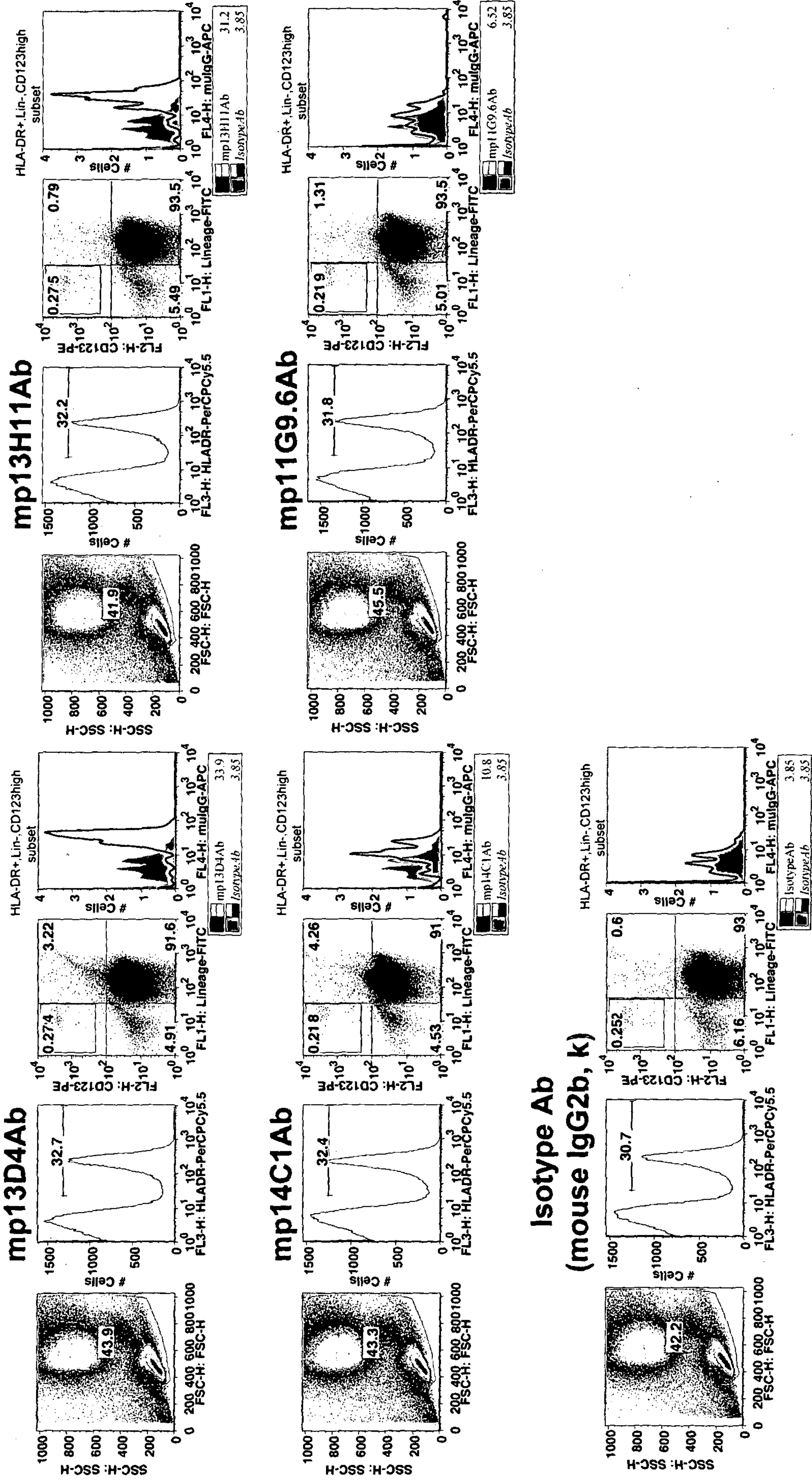


FIG. 18-2



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FIG. 19

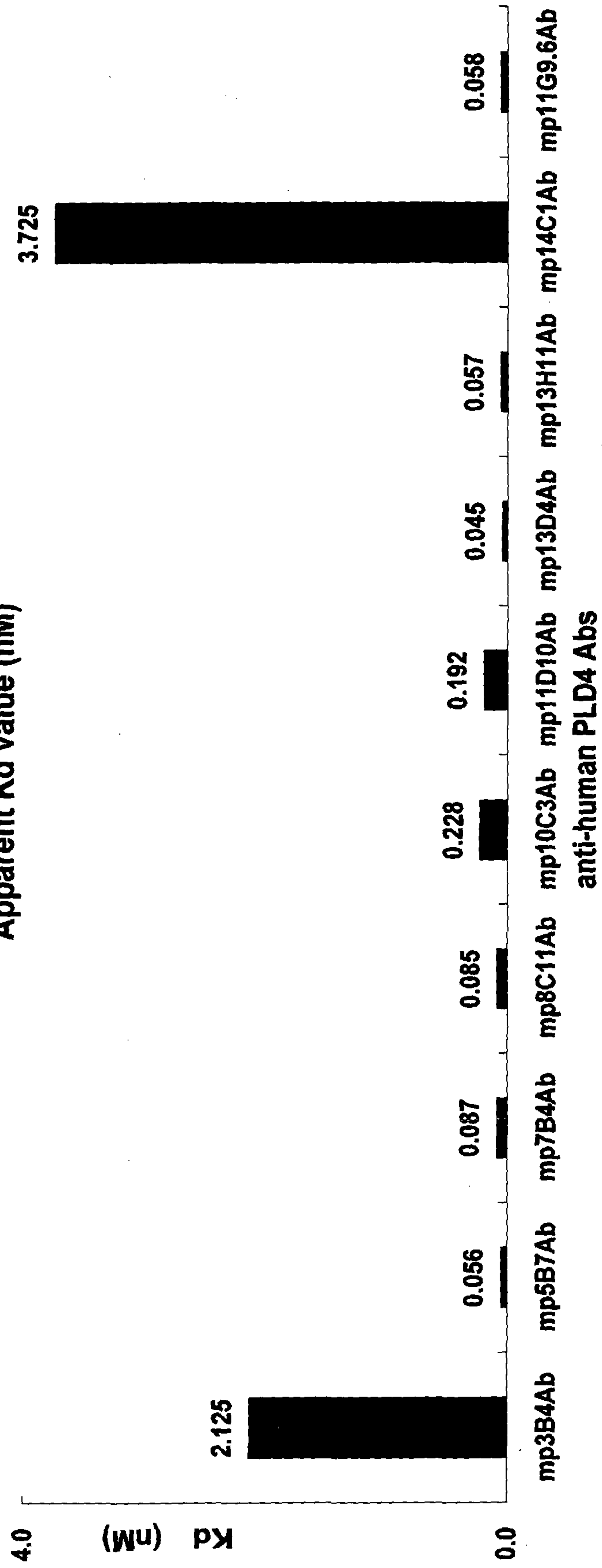
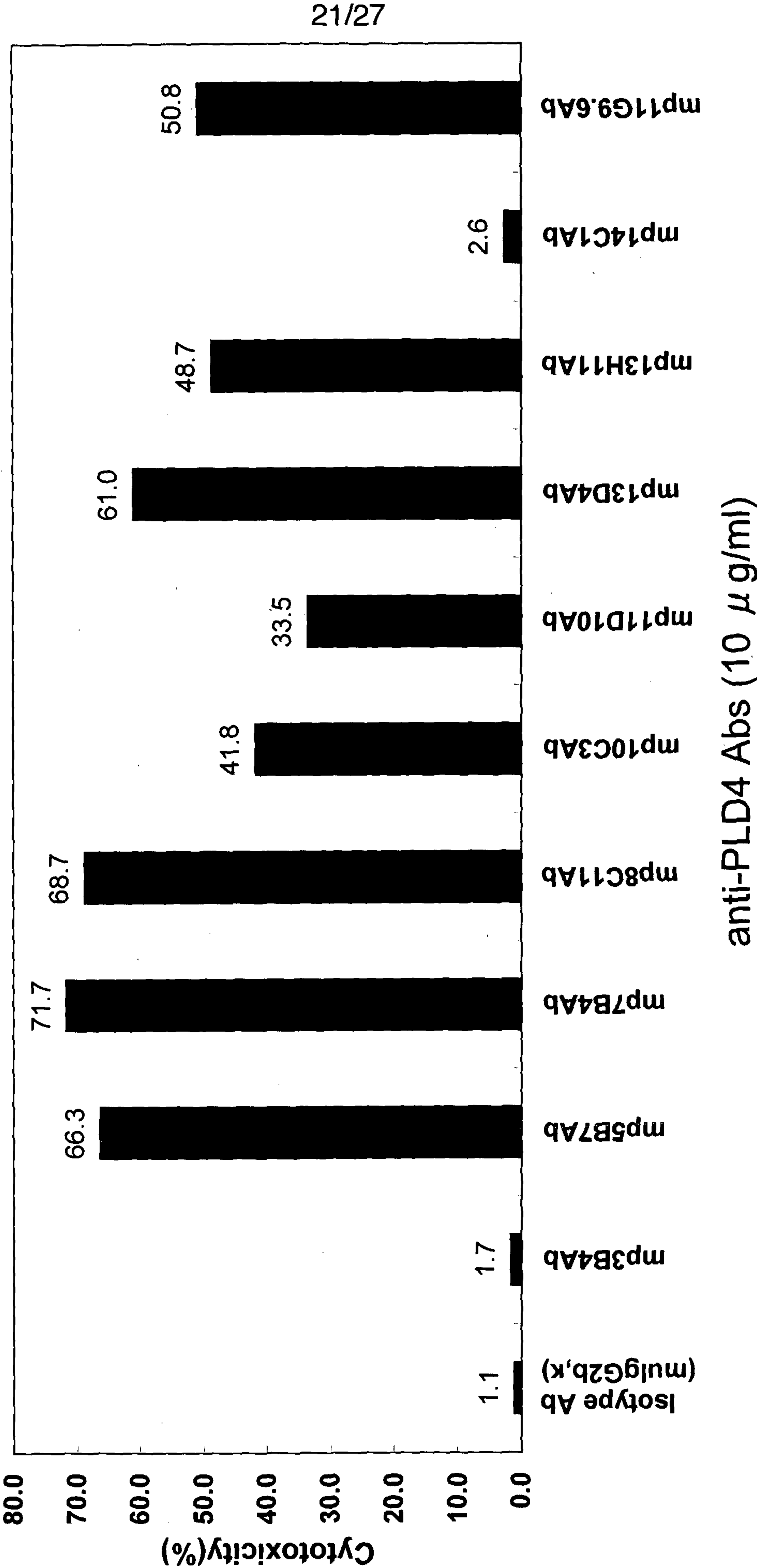
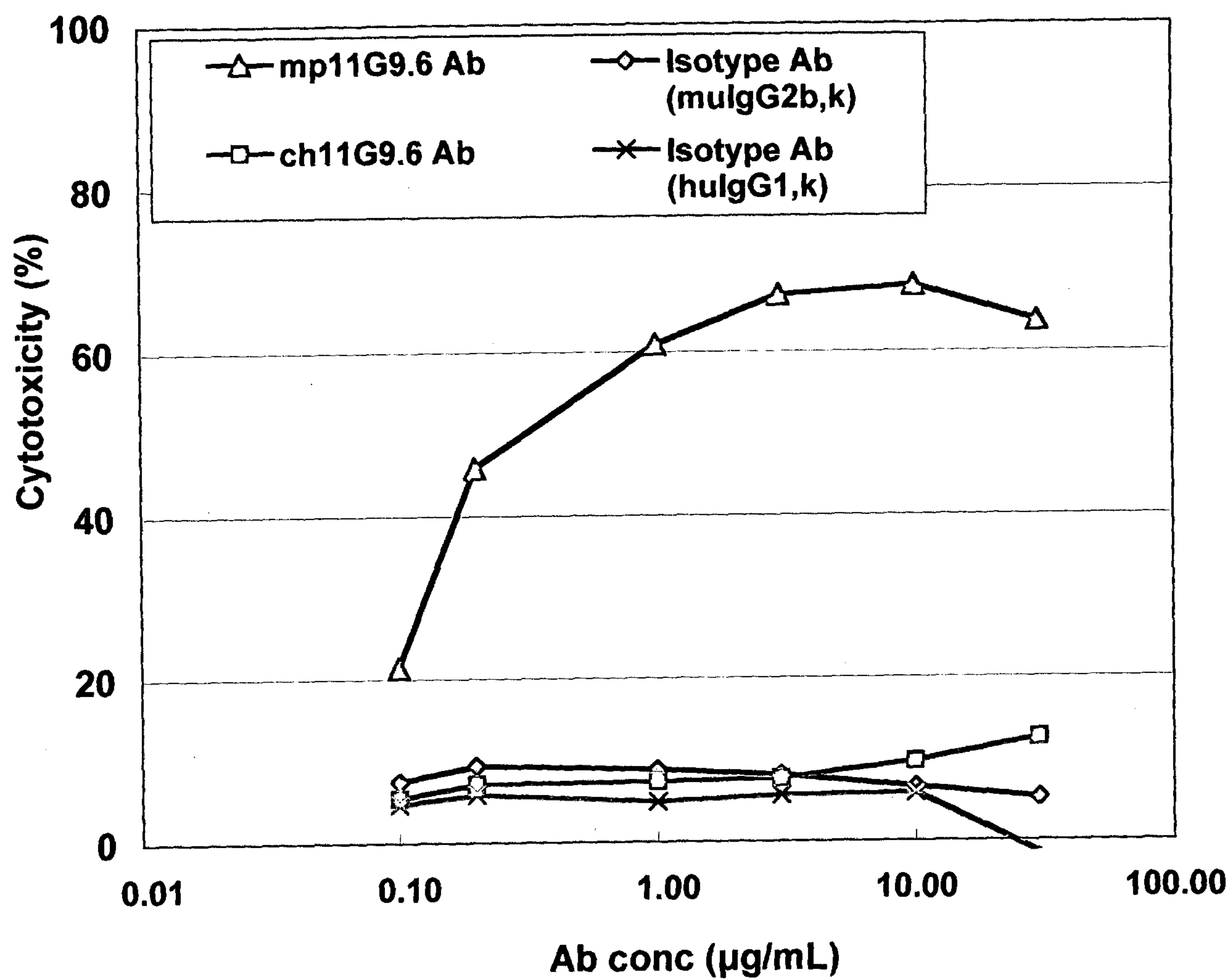
Apparent K_d value (nM)

FIG. 20



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FIG. 21



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FIG. 22

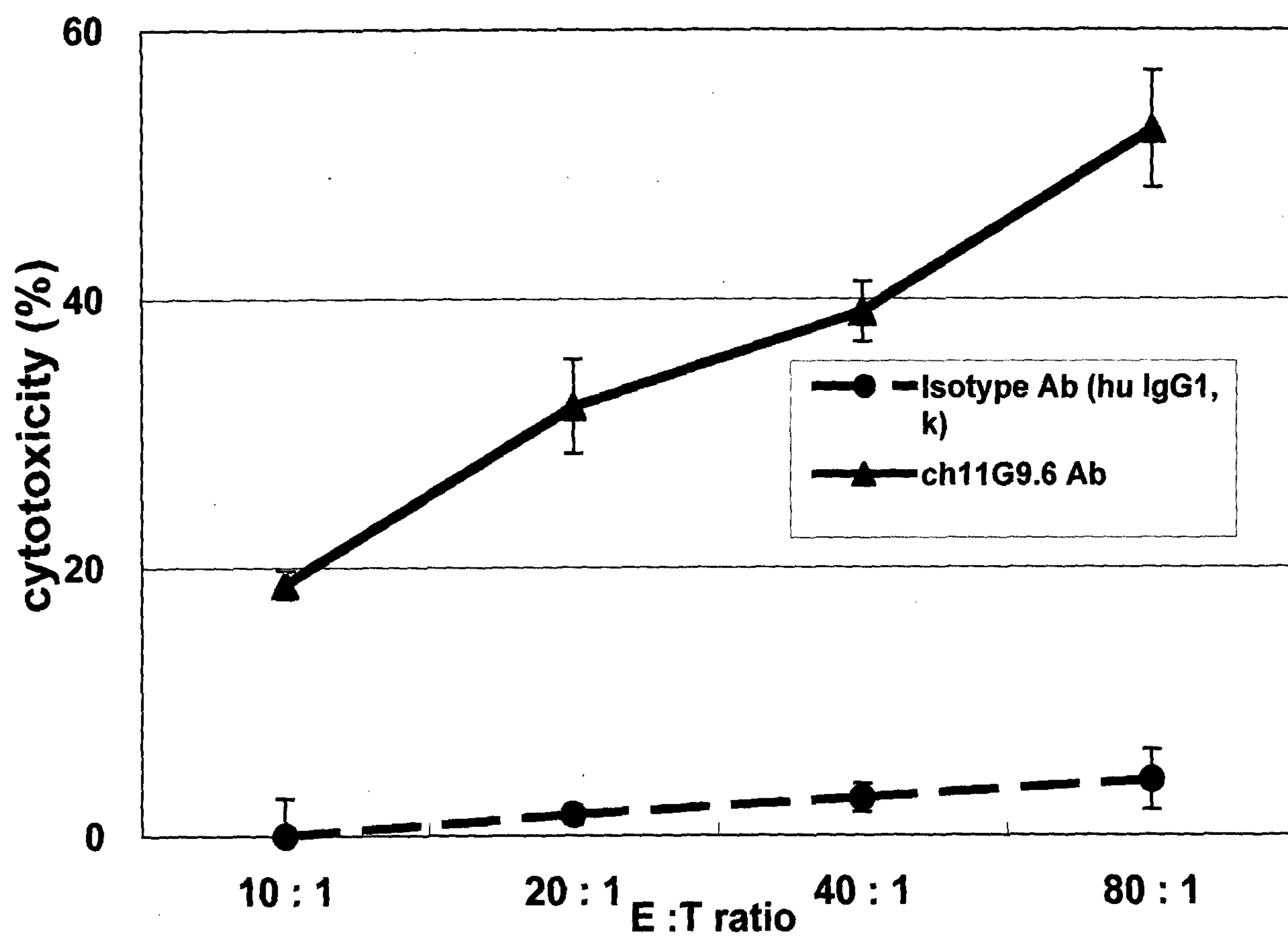
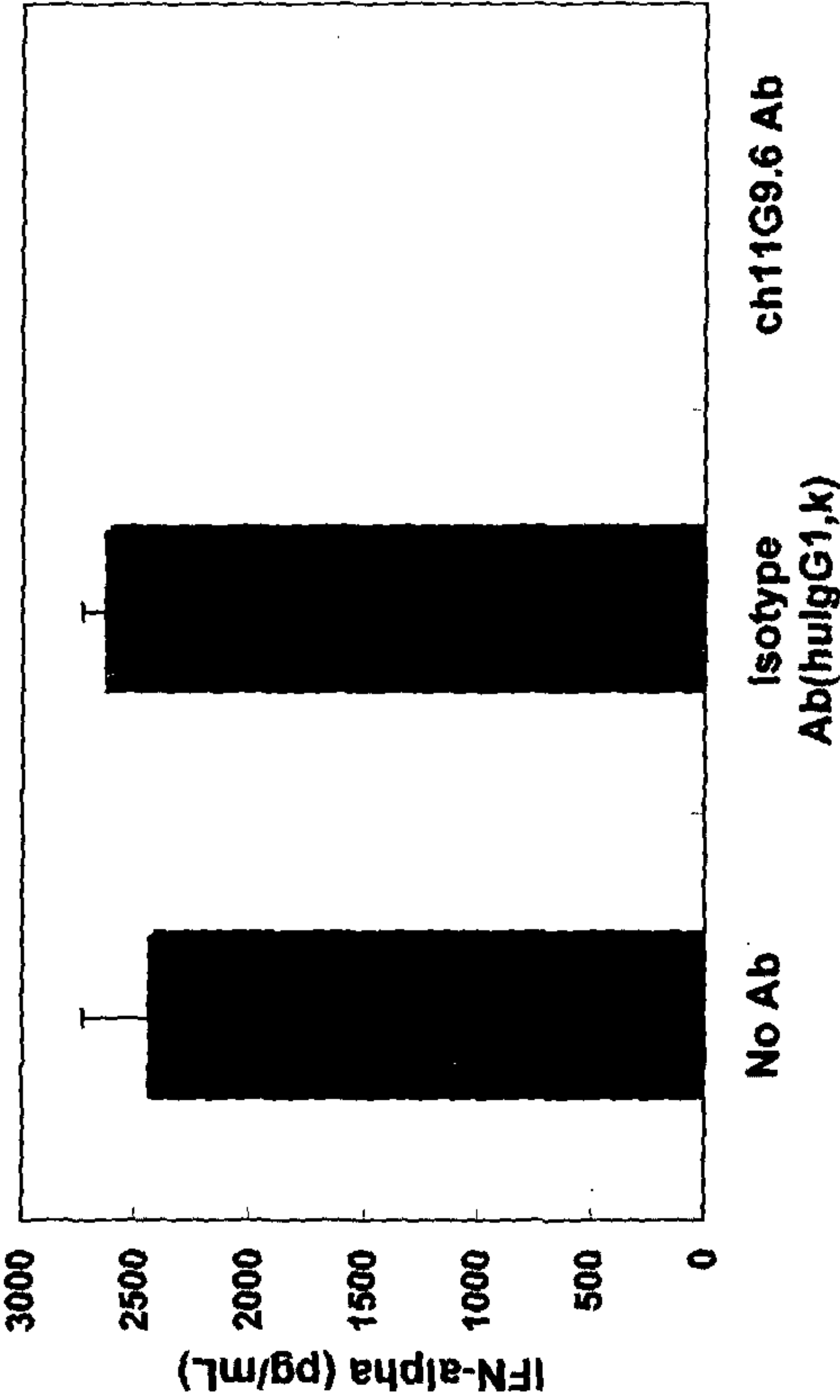
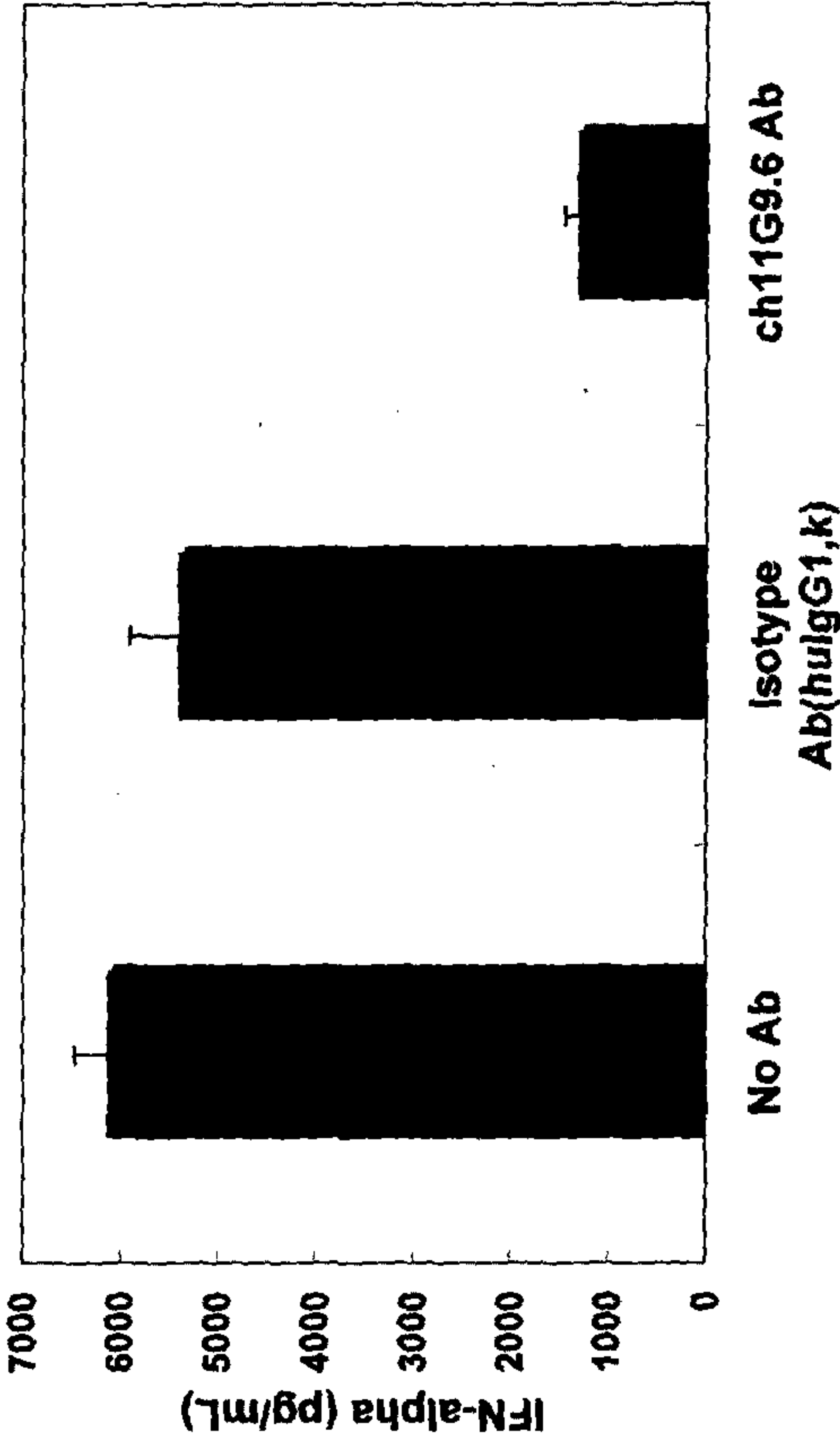


FIG. 23

Healthy donor #1



Healthy donor #2



Healthy donor #3

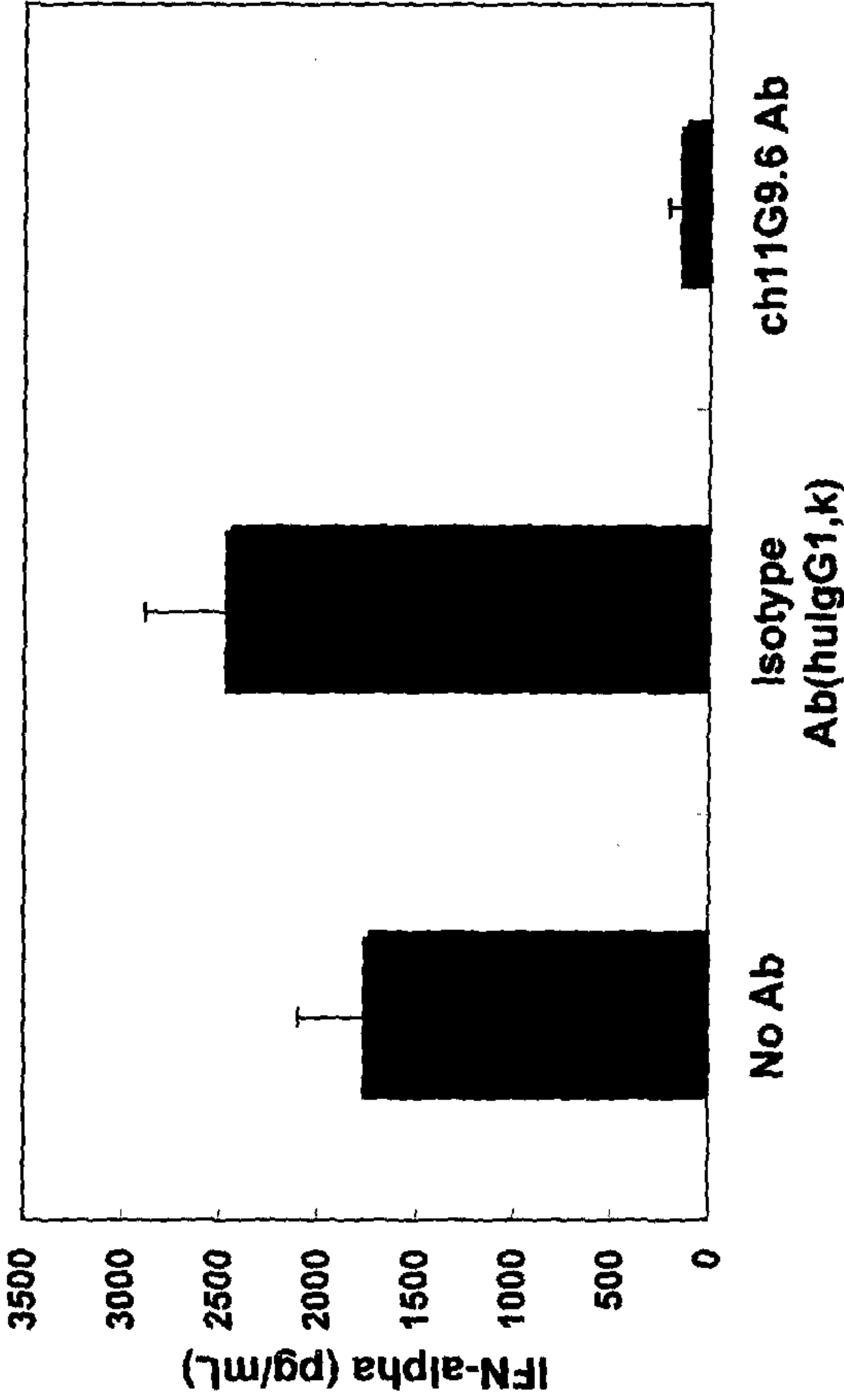
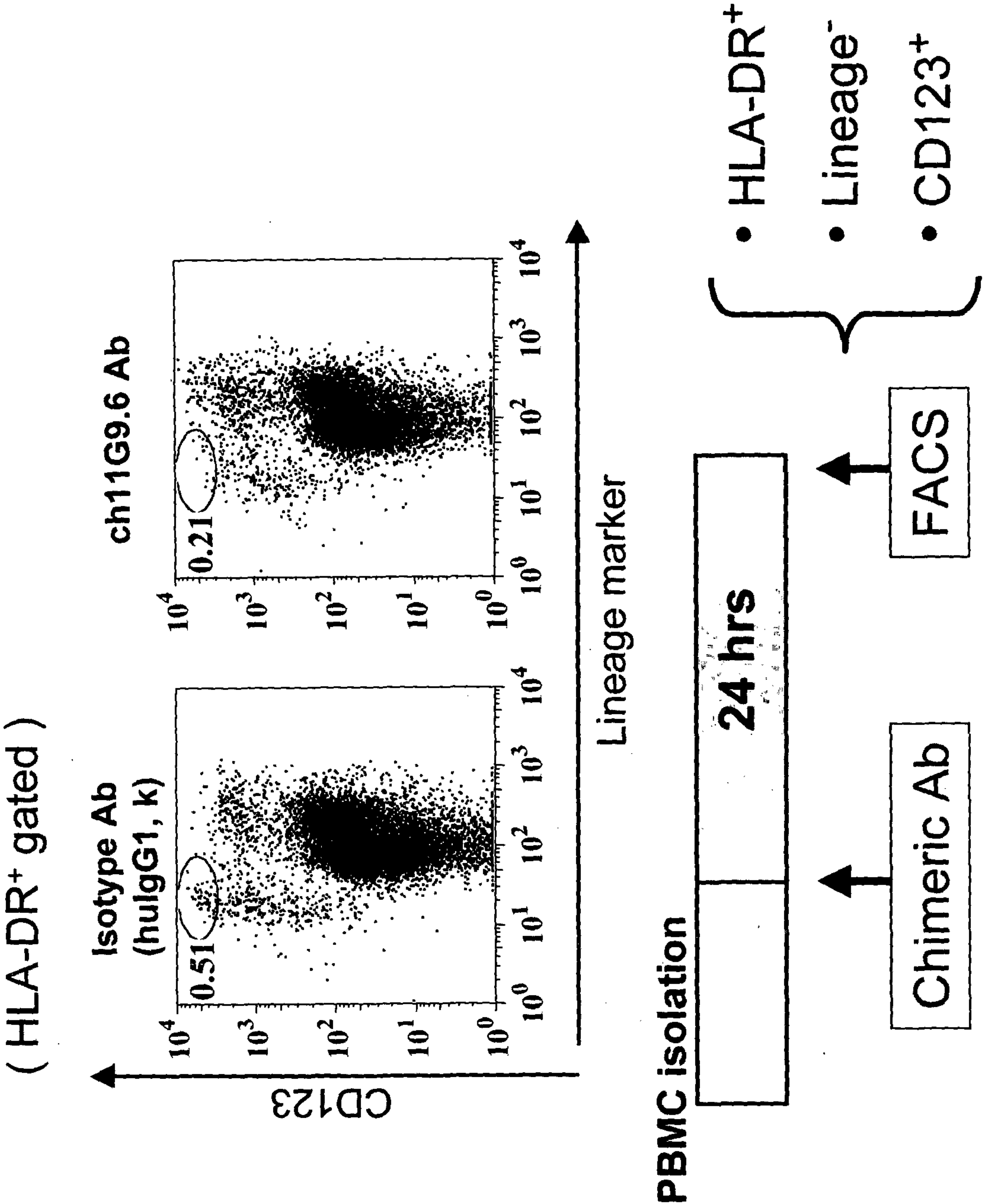


FIG. 24



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FIG. 25

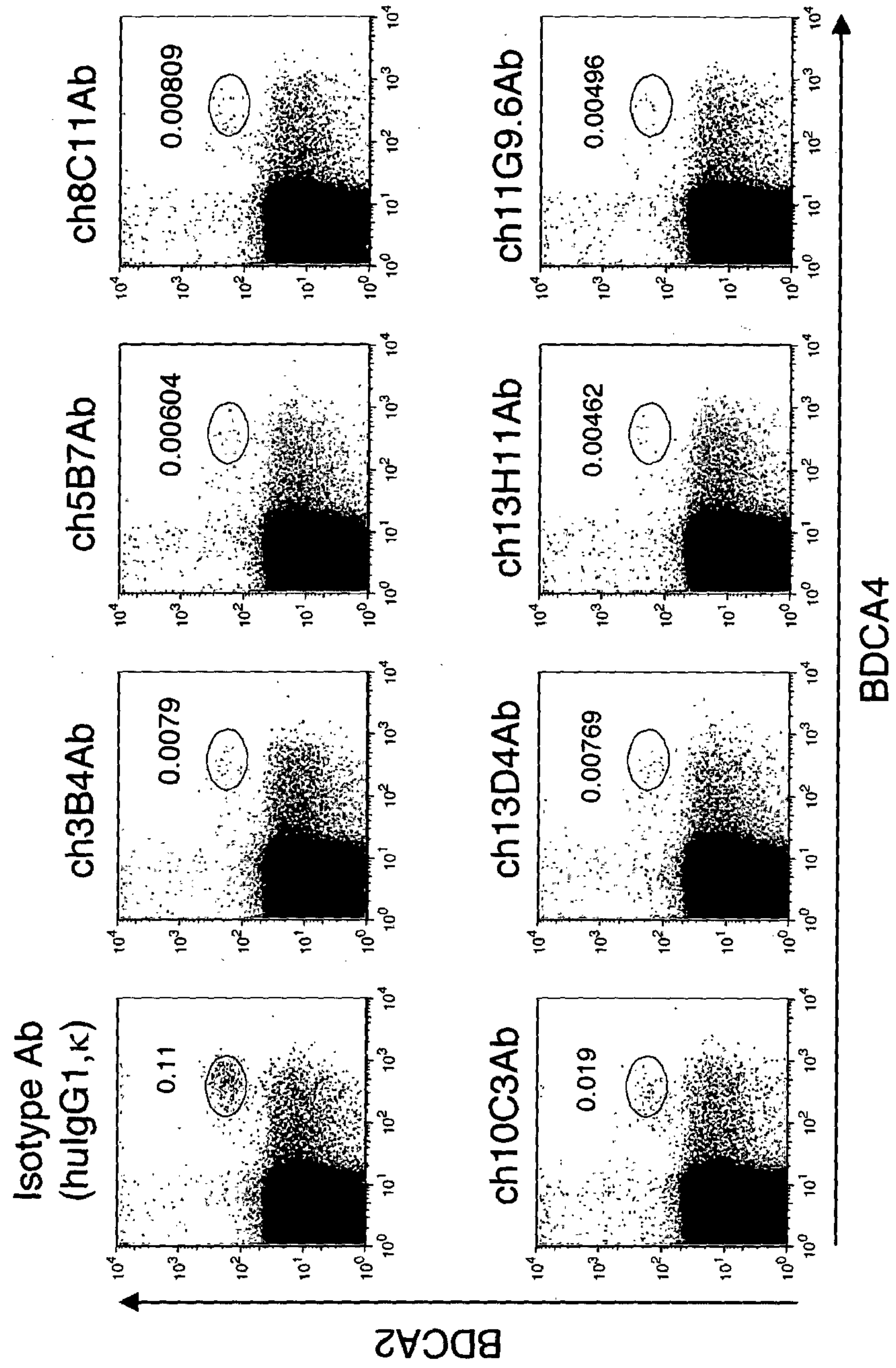


FIG. 26

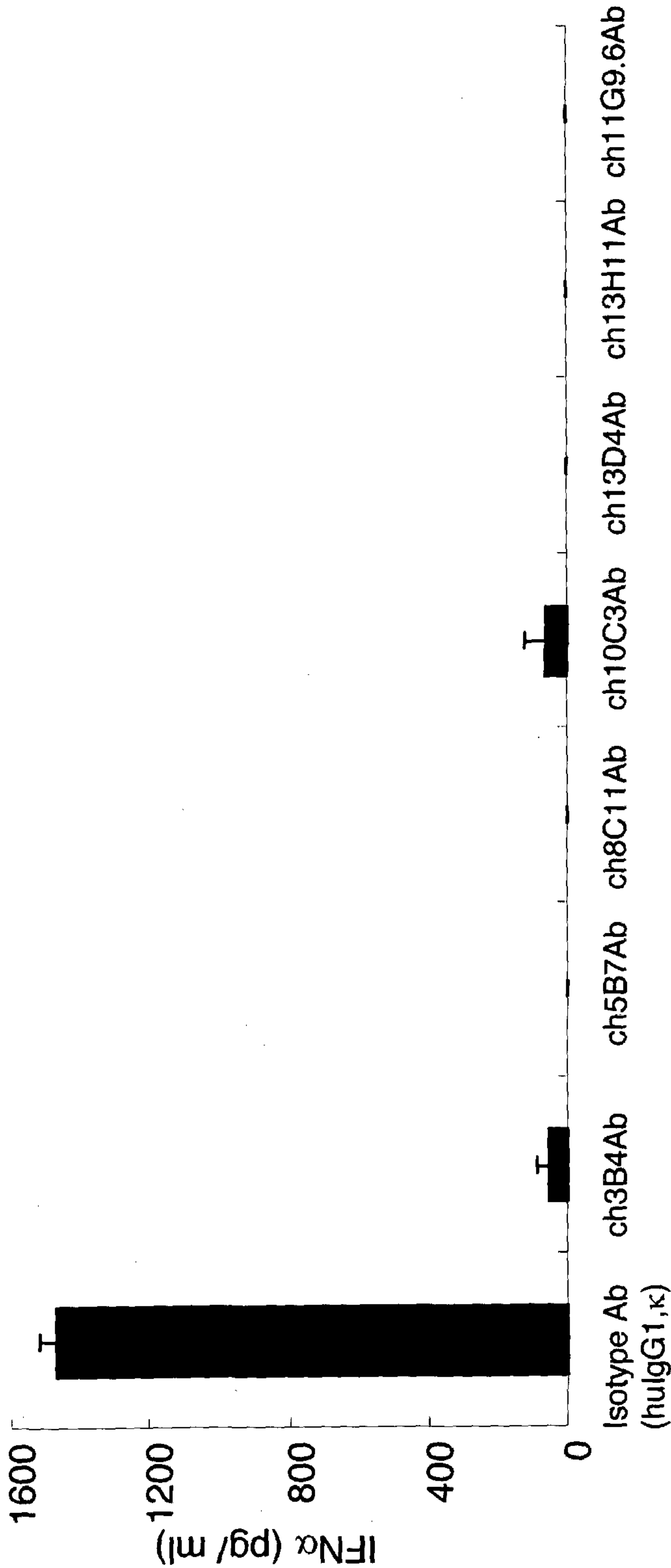


FIG. 26

