Detect NKT Cells with CD1d Tetramers

Available for human and mouse CD1d

Natural killer T (NKT) cells are implicated in the regulation of immune responses associated with a broad range of diseases, and seem to be essential for several aspects of immunity. They represent a unique lymphocyte population that co-express NK cell markers and a semi-invariant T cell receptor. When stimulated with CD1d-restricted glycolipid antigen, NKT cells produce large amounts of Th1-type and/or Th2-type cytokines that lead to downstream activation of dendritic cells, NK cells, B cells and T cells. The dysfunction or deficiency of NKT cells has been shown to lead to the development of autoimmune diseases (such as diabetes or atherosclerosis) and cancers, and they have also been implicated in the disease progression of human asthma .

Human and Mouse CD1d Tetramers Pre-loaded for Added Convenience

ProImmune is the only commercial source worldwide for fluorescently labeled human and mouse CD1d tetramers. The tetramers are available pre-loaded with alpha-Galactosyl Ceramide• (α-GalCer) or empty for loading with ligand of choice.

Human and Mouse CD1d Tetramer Negative Control

ProImmune supplies human and mouse fluorescent CD1d negative control tetramer, which is mock-loaded with carrier only (no ligand loaded) and will not bind to NKT cells. The use of a negative control reagent in conjunction with a ligand loaded CD1d tetramer will allow low frequency positive populations to be accurately quantified, (note: the negative control CD1d tetramer cannot be loaded with ligand).

Experimental Data: Human CD1d Tetramer

Human CD1d tetramer pre-loaded with α-GalCer•, and negative control tetramer, APC labeled and tested with PBMCs.

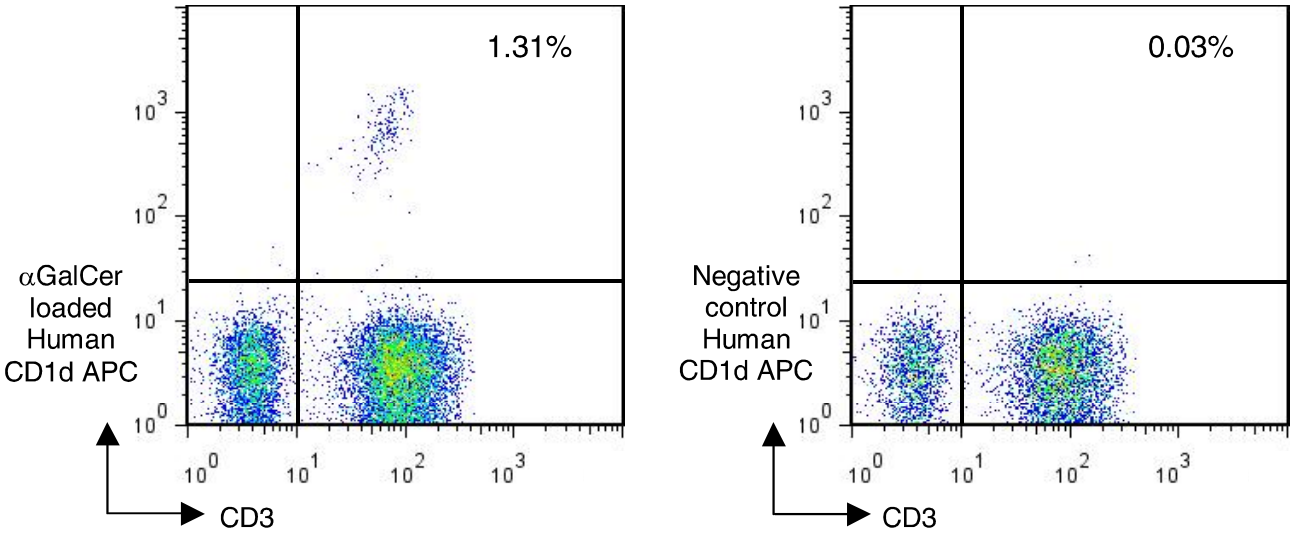

Figure 1: 1 x 10^6 PBMCs were incubated with 1 test (0.5 µl) APC labeled, α–GalCer loaded human CD1d tetramer (left plot), or 1 test (0.5 µl) APC labeled, negative control human CD1d tetramer (right plot) for 30 minutes at 4°C. Following a wash step the cells were incubated at 4°C for 20 minutes with anti-CD3 FITC and anti-CD19 PE in 50 µl total volume. Following two further washes the cells were acquired and analyzed by flow cytometry. Non-specific staining was eliminated from the plot by gating on CD19 negative cells before plotting CD1d tetramer vs CD3.

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Experimental Data: Mouse CD1d Tetramer

Dr. Markus Skold and Dr. Sam Behar, Harvard Medical School (USA), tested ProImmune's mouse CD1d R-PE-labeled tetramer with splenocytes from a naïve B6 mouse depleted of B cells. The tetramer was used empty or loaded with alpha-GalCer•. Cells were also stained with anti-CD4-Alexa488 and anti-CD3-PerCP monoclonal antibodies and gated on live, CD3 positive cells.

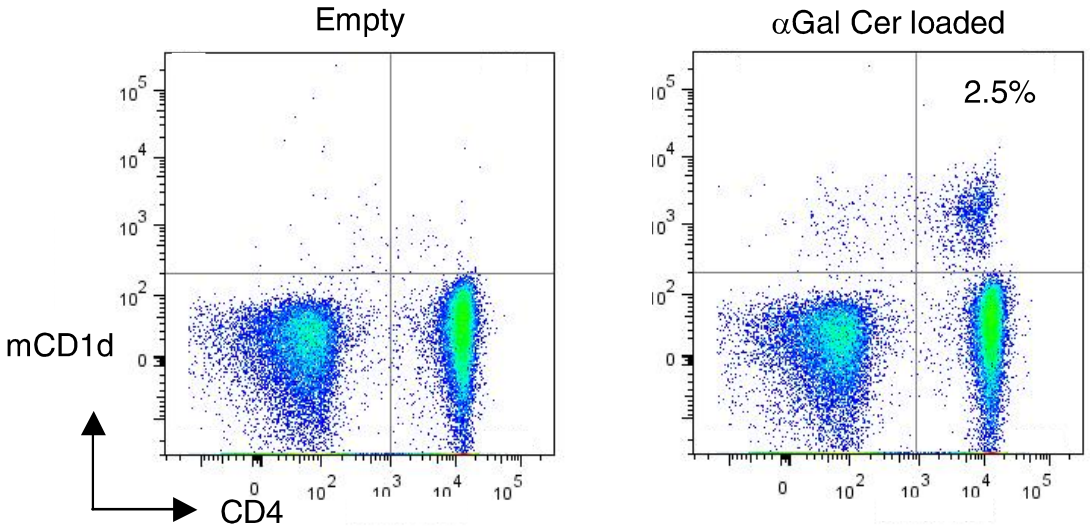

Figure 2: In order to reduce background staining, splenocytes were depleted of B cells using CD19 microbeads (Miltenyi Biotec). The procedure used 3 x 10^5 cells per stain. Cells were incubated with 2.4G2 monoclonal antibody at 25µg/ml in 50µl per sample, at 4°C for 15 minutes in order to block Fc receptors. Following a wash step, cells were incubated with one test (2µl) of CD1d tetramer for 30 minutes. The cells were then incubated at 4°C for 20 minutes with anti-CD4-Alexa488 and anti-CD3-PerCP monoclonal antibodies in 50µl total volume. Following two further washes, the cells were acquired and analyzed by flow cytometry.

Selected Publications citing the use of ProImmune's CD1d tetramers

Normal development and function of invariant NKT cells in mice with iGb3 deficiency.

Porubsky et al. (2007) PNAS 104: 5977-5982 PubMedID: 17372206
In this study, Porubsky et al used mouse CD1d tetramer loaded with alpha-GalCer to determine iNKT cell frequencies in thymus, spleen and liver of mice deficient in isoglobotrihexosylceramide (iGb3) synthase. The study set out to discover whether iGb3 is a physiologically relevant selecting ligand in the mouse, as it had previously been indirectly implicated as the endogenous ligand responsible for positive iNKT selection in the thymus.

Invariant and non-invariant NKT cells exert opposite regulatory functions on the immune response during murine schistosomiasis.

Mallevaey et al. (2007) Infection and Immunity 75:2171-2180 PubMedID: 17353286
A group at the Institut Pasteur in Lille used ProImmune's CD1d tetramer to analyze the proportions of iNKT-cell and non-iNKT-cell populations in two different strains of NKT-cell-deficient mice before and after infection with the parasite Schistosoma mansoni.

Dicer-dependent microRNA pathway controls invariant NKT cell development..

Fedeli et al. (2009), J Immunology, 183: 2506-2512 PubMed ID: 19625646
ProImmune's mouse CD1d tetramers were used to stain cells from conditional knockout mice with one or both Dicer genes deleted. It was shown that deletion of the Dicer gene resulted in a markedly lower frequency of iNKT cells in the thymus and peripheral organs than in wild type littermates, whilst T cell lineages were unaffected by Dicer deletion.

Detect NKT Cells with CD1d Tetramers

CD1d Tetramers

CD1d molecules are non-classical MHC molecules that are characterized as non-polymorphic, conserved among species and possessing narrow, deep, hydrophobic ligand binding pockets. These binding pockets are capable of presenting glycolipids and phospholipids to Natural Killer T (NKT) cells. NKT cells represent a unique lymphocyte population that co-express NK cell markers and a semi-invariant T cell receptor (TCR). They are implicated in the regulation of immune responses associated with a broad range of diseases.

The best characterized CD1d ligand is α-Galactosyl Ceramide• (α-GalCer), originally derived from marine sponge extract. Presentation of α-GalCer by CD1d molecules results in NKT cell recognition and rapid production of large amounts of IFN-gamma and IL-4, bestowing α-GalCer with therapeutic efficacy. More recently, the lysosomal sphingolipid isoglobotrihexosylceramide (iGb3) has been identified as a CD1d ligand. This endogenous sphingolipid is thought to be responsible for NKT cell development.

ProImmune provides fluorescently labeled human and mouse CD1d tetramers loaded with α-GalCer, or empty for loading with the ligand of choice by the user. Tetrameric CD1d-lipid complexes bind to TCRs of NKT cells of a particular specificity (as determined by the lipid ligand used), allowing identification and enumeration of antigen-specific CD1d-restricted NKT cells by flow cytometry. Additional co-staining for intracellular cytokines such as IFN gamma or IL-2 and/or surface markers e.g. CD69 can yield functional data for the antigen-specific subset.

CD1d Tetramer Product Codes

Code	Human CD1d	Code	Mouse CD1d
D000-2A	50 tests R-PE, Empty	E000-2A	50 tests R-PE, Empty
D000-2B	150 tests R-PE, Empty	E000-2B	150 tests R-PE, Empty
D000-2C	500 tests R-PE, Empty	E000-2C	500 tests R-PE, Empty
D000-4A	50 tests APC, Empty	E000-4A	50 tests APC, Empty
D000-4B	150 tests APC, Empty	E000-4B	150 tests APC, Empty
D000-4C	500 tests APC, Empty	E000-4C	500 tests APC, Empty
D001-2A	50 tests R-PE, Loaded with α-GalCer•	E001-2A	50 tests R-PE, Loaded with α-GalCer•
D001-2B	150 tests R-PE, Loaded with α -GalCer	E001-2B	150 tests R-PE, Loaded with α -GalCer
D001-2C	500 tests R-PE, Loaded with α -GalCer	E001-2C	500 tests R-PE, Loaded with α -GalCer
D001-4A	50 tests APC, Loaded with α -GalCer	E001-4A	50 tests APC, Loaded with α -GalCer
D001-4B	150 tests APC, Loaded with α -GalCer	E001-4B	150 tests APC, Loaded with α -GalCer
D001-4C	500 tests APC, Loaded with α -GalCer	E001-4C	500 tests APC, Loaded with α -GalCer
D002-2A	50 tests R-PE, Negative Control	E002-2A	50 tests R-PE, Negative Control
D002-2B	150 tests R-PE, Negative Control	E002-2B	150 tests R-PE, Negative Control
D002-2C	500 tests R-PE, Negative Control	E002-2C	500 tests R-PE, Negative Control
D002-4A	50 tests APC, Negative Control	E002-4A	50 tests APC, Negative Control
D002-4B	150 tests APC, Negative Control	E002-4B	150 tests APC, Negative Control
D002-4C	500 tests APC, Negative Control	E002-4C	500 tests APC, Negative Control

• The alpha GalCer molecule and derivatives thereof are covered by US Patent No.5,936,076, which is owned by Kirin Pharma. The alpha GalCer molecule is licensed to Funakoshi Co. Ltd. for research use worldwide.

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