CD1d molecules are highly-conserved non-classical major histocompatibility complex (MHC) molecules that are characterized as non-polymorphic 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), and 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-γ and IL-4, bestowing α-GalCer with therapeutic efficacy.

**α-GalCer Loaded Recombinant CD1d Tetramer:**

ProImmune’s fluorescent-labeled CD1d tetramers loaded with α-GalCer are used to identify Natural Killer (NK) T cells. CD3⁺ NKT cells stained with CD1d Tetramer can be analyzed by flow cytometry and the frequency of antigen-specific NKT cells determined. Additional co-staining for intracellular cytokines (e.g. IFNγ / IL-2) or surface markers (e.g. CD69 / CD45RO) can provide additional functional data on the antigen-specific sub-set.

**For Research Use Only. Not for use in therapeutic or diagnostic procedures.**

**Test Volume:** 0.5 μl / test.

**Test Specification:** One test contains sufficient reagent to stain approximately 1 × 10⁶ cells. We recommend that the user titrate the reagent to determine the optimum amount to use in their specific application.

**Concentration/Formulation:** The CD1d Tetramer concentration is approximately 1μM. The Tetramer is loaded with 0.2 mg/ml α-GalCer solubilized in PBS + 0.5% Tween-20, and supplied in PBS stabilized with 1% BSA and 0.01% sodium azide.

**Storage Condition:** 4°C. Protect from light. **Do not freeze.**

**Shelf Life:** 6 months if stored as instructed above.

**Fluorochrome:** R-phycoerythrin (R-PE) excites at 480, 565 nm and emits at 578 nm (FL-2).

**Hazards:** This reagent is formulated in 0.01% sodium azide. Under acid conditions the toxic compound hydrazoic acid may be released. Compounds containing sodium azide should be flushed with running water while being discarded.

**Quality Control Assay Results**

**Appearance:** Clear, pale pink solution

**Protein Characterization:** Passed

**MHC Conformation Immunoassay:** Passed

**Released by:** (Date as per product label above)
**Cellular Staining Protocol**

**Additional materials required:** Wash Buffer (0.1% sodium azide, 0.1% BSA in PBS), Fix Solution (1% fetal calf serum, 2.5% formaldehyde in PBS), anti-CD3 antibody, anti-CD19 antibody.

1. Allocate 1-2×10^6 lymphoid cells (PBMCs or splenocytes) per staining condition. Allocate only 2-5×10^5 cells per staining condition when using NKT cell clones or lines, due to the higher frequency of antigen-specific NKT cells.
2. Wash the cells with 2 ml Wash Buffer and resuspend them in the residual volume (~50 μl). Keep tubes chilled on ice for all subsequent steps.
3. Add one test (0.5 μl) of fluorescently-labeled CD1d tetramer to the cells and mix well.
4. Incubate samples on ice for 30 minutes, shielded from light.
5. Wash the cells with 2 ml Wash Buffer and resuspend them in the residual volume.
6. Add anti-CD3 and anti-CD19 antibodies to the cells and mix well. (Use of an anti-CD19 antibody enables non-specific staining of B cells to be excluded from the cytometry analysis.)
7. Incubate samples on ice for 20-30 minutes, shielded from light.
8. Wash the cells twice with 2 ml wash buffer and resuspend thoroughly before adding 200 μl Fix Solution. Store them in Fix Solution in the dark until analysis.

Tetramer-positive cells are most conveniently viewed by gating first on live, CD19-negative lymphoid cells and then analyzing on a two-color plot showing CD3 on the x-axis and tetramer on the y-axis.

**Protocol Optimization**

The following guidelines will help you optimize your protocol for the best possible results:

**Setting the live lymphocyte gate** It is important to ensure that the forward-scatter (FSC) and side-scatter (SSC) gates are set correctly on the cell population of interest. This is to ensure that dead cells, cell aggregates and cell debris are excluded from the fluorescence data.

**Titrating the CD1d Tetramer** Carry out a range of doubling dilutions from 1 test per 1×10^6 cells down to 1/16 test per 1×10^6 cells, in order to determine the optimum amount of Pentamer reagent to use in your specific application.

**Anti-CD3 antibody** Investigate the effect of selecting different antibody clones or titrating the anti-CD3 antibody.

**Positive control** Pro5® MHC Pentamers should be tested against a specific NKT cell line (or clone). Be sure to use NKT cells that have not been recently stimulated as this has been shown to cause down-regulation of NKT cell receptors. If an NKT cell line is not available, use PBMCs from a known positive donor - the frequency of positive cells will be much lower and therefore sufficient events must be collected to ensure a clear result.

**Negative Control** To control for non-specific staining it is also useful to stain T cells with the CD1d Negative Control Tetramer (Code D002 or E002), which are mock-loaded with carrier only (no ligand loaded) and will not bind to NKT cells. The use of this negative control reagent in conjunction with a ligand-loaded CD1d Tetramer (e.g. α-GalCer) will allow low frequency positive populations to be accurately quantified.

The figure on the left shows a cell sample stained with Negative Control Tetramer (Code D002 or E002). The figure on the right shows the same cells stained with the α-GalCer loaded CD1d Tetramer. A population of CD3⁺ Tetramer-positive NKT cells is clearly visible in the upper right quadrant. Non-specific staining was eliminated from the plot by gating on CD19- cells before plotting CD3 vs. CD1d Tetramer.

© Copyright Proimmune Limited 2003-2016. All Rights Reserved.

PS_DE001-RPE (CD1d Tetramer α-GalCer Loaded (R-PE Labeled) Version 1.1