

**PRODUCT SHEET**CD1d Tetramer R-PE Labeled  
Negative Control:

- D002-2X
- E002-2X

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  $\alpha$ -galactosyl ceramide ( $\alpha$ -GalCer), originally derived from marine sponge extract. Presentation of  $\alpha$ -GalCer by CD1d molecules results in NKT cell recognition and rapid production of large amounts of IFN- $\gamma$  and IL-4, bestowing  $\alpha$ -GalCer with therapeutic efficacy.

**Negative Control  
Recombinant CD1d  
Tetramer:**

ProImmune's fluorescent-labeled CD1d negative control tetramers are mock-loaded with carrier only (no ligand loaded) and will not bind to NKT cells. Use of this negative control reagent in conjunction with a ligand-loaded CD1d Tetramer (e.g.  $\alpha$ -GalCer) will allow low frequency positive populations to be accurately quantified.

Please note that the negative control CD1d Tetramer cannot be reloaded with ligand by the end-user. ProImmune can provide a specific empty CD1d tetramer for this purpose (Catalog code D000 or E000).

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

**Test Volume:** 0.5  $\mu$ l / test.

**Test Specification:** One test contains sufficient reagent to stain approximately  $1 \times 10^6$  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 1  $\mu$ M. The Tetramer is 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  $1-2 \times 10^6$  lymphoid cells (PBMCs or splenocytes) per staining condition. Allocate only  $2-5 \times 10^5$  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  $\mu$ l). Keep tubes chilled on ice for all subsequent steps
3. Add one test (0.5  $\mu$ l) 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  $\mu$ l 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:

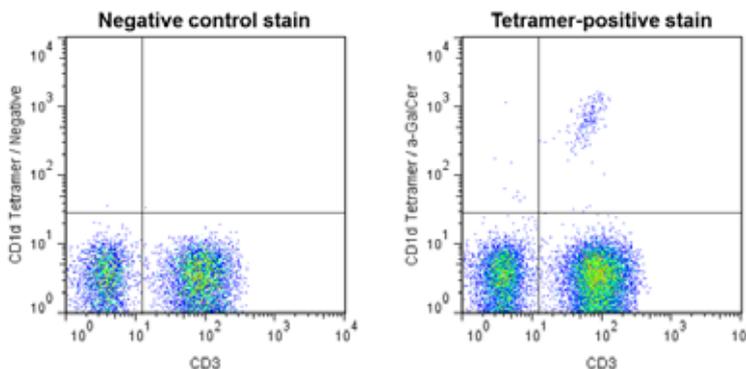
**Setting the live lymphocyte gate** It is important to ensure that the forward-scatter (FSC) and side-scatter (SSC) gates are set correctly on the cell population of interest. This is to ensure that dead cells, cell aggregates and cell debris are excluded from the fluorescence data.

**Titrating the CD1d Tetramer** Carry out a range of doubling dilutions from 1 test per  $1 \times 10^6$  cells down to 1/16 test per  $1 \times 10^6$  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<sup>®</sup> 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.  $\alpha$ -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  $\alpha$ -GalCer loaded CD1d Tetramer. A population of CD3<sup>+</sup> Tetramer-positive NKT cells is clearly visible in the upper right quadrant. Non-specific staining was eliminated from the plot by gating on CD19<sup>-</sup> cells before plotting CD3 vs. CD1d Tetramer.