

Technical Data Sheet

DimerX I: Recombinant Soluble Dimeric Mouse CD1d:Ig Fusion Protein

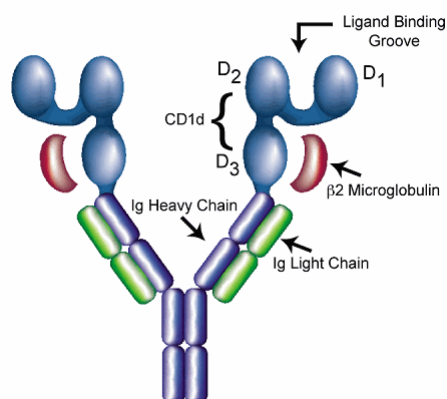
Product Information

| | |
|-------------------------|--|
| Material Number: | 557599 |
| Size: | 0.25 mg |
| Concentration: | 0.5 mg/ml |
| Isotype: | Mouse IgG1, λ |
| Storage Buffer: | Aqueous buffered solution containing $\leq 0.09\%$ sodium azide. |

Description

The CD1d:Ig fusion protein consists of the extracellular major histocompatibility complex (MHC) class I-like domains of the mouse CD1d molecule fused with the VH regions of mouse IgG1 (see figure). Like MHC class I molecules, the CD1d noncovalently associates with $\beta 2$ Microglobulin ($\beta 2$ M). For this reason, BD Pharmingen DimerX consists of recombinant CD1d:Ig and $\beta 2$ M. Recombinant CD1d molecules, like the DimerX fusion protein, are useful for studying Natural Killer T (NKT)-cell function by immunofluorescent staining and flow cytometric analysis of antigen-specific NKT cells.

The mouse *CD1d1* gene encodes a non-polymorphic cell-surface protein that plays a role in antigen presentation to CD1d-restricted NKT cells. Like the MHC class I molecules, CD1d associates noncovalently with $\beta 2$ M and is capable of binding and presenting lipid antigens. While the natural ligand for CD1d is presently unknown, it is well documented that CD1d can bind and present the glycolipid, α -galactosyl ceramide (α -GalCer*), a glycosphingolipid from the marine sponge. Glycosylphosphatidylinositol (GPI) has also been identified as a major CD1d-associated component. Antigenic glycolipids such as α -GalCer associated with the CD1d molecule are presented and specifically recognized by NKT cells expressing a highly conserved TCR, V α 14Ja281, paired with diverse β chains such as V β 8.2 and V β 7. Mouse CD1d:Ig shows differential staining intensities on V α 14Ja281+ cells, based on the V β chain present, with V β 8.2+ cells predominantly showing higher intensities than V β 7+ cells. The function and lineage marker expression of NKT cells and their interaction with CD1d has been recently reviewed.



Schematic representation of the CD1d:Ig dimeric protein.

Preparation and Storage

Store undiluted at 4°C.

The mouse CD1d:Ig fusion protein was expressed together with mouse $\beta 2$ M in the mouse plasmacytoma cell line, J558L (ATCC TIB-6). The CD1d:Ig and $\beta 2$ M polypeptide chains are associated noncovalently as a consequence of their coexpression within J558L cells. The CD1d:Ig fusion protein was purified from tissue culture supernatant by affinity chromatography. The purity of the preparation was confirmed by SDS-PAGE. The CD1d:Ig has been tested by ELISA to verify the fusion protein.

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Application Notes

Application

| | |
|----------------|---------------------------|
| ELISA | Routinely Tested |
| Flow cytometry | Tested During Development |

Recommended Assay Procedure:

It is necessary to load the CD1d portions of the dimeric protein with a relevant antigen of interest prior to immunofluorescent staining of NKT cells. CD1d:Ig complexes are effectively loaded by incubation with excess relevant (specific) or irrelevant (control) antigens (see Protocol 1). Antigen-loaded CD1d:Ig may be used for immunofluorescent staining (see Protocol 2). **Since applications vary, each investigator must determine dilutions appropriate for individual use.**

Protocol 1: Antigen Loading of CD1d:Ig Dimeric Protein

Several antigen-loading protocols have been described in the literature. The method recommended at BD Biosciences Pharmingen involves passive loading of excess antigen in solution with CD1d:Ig protein. We have found that passive loading works particularly well in the case of high-affinity antigens. For lower-affinity antigens, an increase in the molar ratio of antigen to dimer protein may improve loading, as determined by flow cytometric analysis based on results for other BD Pharmingen DimerX products. It is suggested that for each antigen, parameters such as the dose of CD1d:Ig per million cells, molar ratio of antigen to CD1d:Ig, and antigen-loading time be determined empirically by the investigator.

Antigen preparation and loading:

1. The molecular weight (MW) of an antigen of interest will need to be determined. The MW of α -GalCer is 858 daltons.
2. Mix CD1d:Ig protein with specific or control antigen at 10, 20, or 40 molar (M) excess.

The following calculation, using α -GalCer as an example, may be used:

Dg = Molecular Weight of antigen: eg, 858 daltons.

DCD1d = Molecular Weight of CD1d:Ig = 250,000 daltons.

R = desired excess molar ratio, eg, 40.

Mg = micrograms (μ g) antigen of interest.

MCD1d = micrograms (μ g) CD1d:Ig in the reaction. A typical amount of antigen-loaded CD1d:Ig to use for flow cytometry staining is 0.25 to 4 μ g/million cells (test).

$$\text{Mg} = \frac{\text{MCD1d} \times \text{R} \times \text{Dg}}{\text{DCD1d}} = \frac{4 \mu\text{g} \times 40 \times 858 \text{ d}}{250,000 \text{ d}} = 0.55 \mu\text{g}$$

Therefore, one would add 0.55 μ g of antigen and 4 μ g of CD1d:Ig in solution for the optimal antigen loading of CD1d:Ig.

3. Mix antigen and CD1d:Ig together in PBS, pH 7.2, incubate at 37°C overnight. The antigen-loaded CD1d:Ig can be stored at 4°C for up to 1 week.

Protocol 2: Immunofluorescent Staining Protocol

1. Prepare peptide-loaded CD1d:Ig protein staining cocktail by mixing 0.25 - 4 μ g of peptide-loaded CD1d:Ig protein/test with 0.25 - 4 μ g of PE-conjugated A85-1 mAb (anti-mouse IgG1, Cat. No. 550083)/test at a ratio of 1:1 or 1:2 of dimer:A85-1 mAb. Incubate the mixture for 60 minutes at RT, protect from exposure to light.
2. Add 0.25 - 4 μ g of purified mouse IgG1 isotype control mAb A111-3 (Cat. No. 553485)/test to the staining cocktail (see Step 1 above). Incubate the staining cocktail for 30 minutes at RT, protect from exposure to light.
3. Resuspend mouse cells in FACS staining buffer [eg, DPBS, 1% FCS, 0.09% NaN3 or BD Pharmingen™ Stain Buffer (FBS), Cat. No. 554656], containing the appropriate amount of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142), at a concentration of approximately 10e6 cells per 50 μ l. Incubate 10 minutes at 4°C. Add $\sim 1 \times 10^6$ cells per staining tube (e.g., 12 x 75 mm tube, BD Falcon™ Cat. No. 352008).
4. Add 50 μ l FACS buffer containing the optimal per test amount of the staining cocktail to each sample, plus any other cell-surface marker-specific antibodies to be used.
5. Wash cells 2x with 2 ml FACS buffer, centrifuge for 5 minutes at 250 x g, and discard supernatant. Resuspend cell pellet in approximately 0.5 ml staining buffer in a tube appropriate for the flow cytometer.

Protocol 3: Alternative: Immunofluorescent Staining Protocol

1. Resuspend mouse cells in FACS staining buffer [eg, DPBS, 1% FCS, 0.09% NaN3 or BD Pharmingen™ Stain Buffer (FBS), Cat. No. 554656], containing the appropriate amount of Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142), at a concentration of approximately 10e6 cells per 50 μ l. Incubate 10 minutes at 4°C. Add $\sim 1 \times 10^6$ cells per staining tube (e.g., 12 x 75 mm tube, BD Falcon™ Cat. No. 352008).
2. Add 0.25 to 4 μ g of antigen-loaded CD1d:Ig protein to cell suspension. Incubate 60 minutes at 4°C.
3. Wash cells 1x with 2 ml FACS buffer, centrifuge for 5 minutes at 250 x g, and aspirate supernatant.
4. Resuspend cells in 100 μ l FACS buffer containing appropriately diluted fluorescent secondary reagent. We typically use PE-conjugated A85-1 mAb (anti-mouse IgG1, Cat. No. 550083). Incubate 30 - 60 minutes at 4°C.

5. Wash cells 2x with 2 ml FACS buffer, centrifuge for 5 minutes at 250 x g, and discard supernatant. Resuspend cell pellet in approximately 0.5 ml staining buffer in a tube appropriate for the flow cytometer.

*The rights to α -GalCer are owned by Kirin Brewery. The α -GalCer molecule and its derivatives are covered by US Patent No. 5,936,076. There is no implied license hereunder for the use of α -GalCer.

Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|----------------|--|--------|--------|
| 553485 | Purified Mouse IgG1 λ Isotype Control | 0.5 mg | A111-3 |
| 553141 | Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) | 0.1 mg | 2.4G2 |
| 550083 | PE Rat Anti-Mouse IgG1 | 0.1 mg | A85-1 |
| 554656 | Stain Buffer (FBS) | 500 ml | (none) |

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

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