Technical Data Sheet

DimerX I:Recombinant Soluble Dimeric Mouse H-2Ld:Ig Fusion Protein

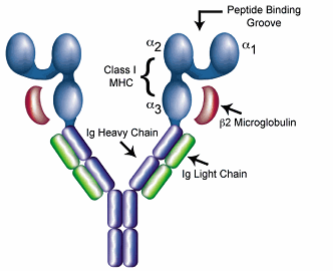
Product Information

Material Number:	**550751**
Size:	0.25 mg
Concentration:	0.5 mg/ml
Isotype:	Mouse IgG1, λ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

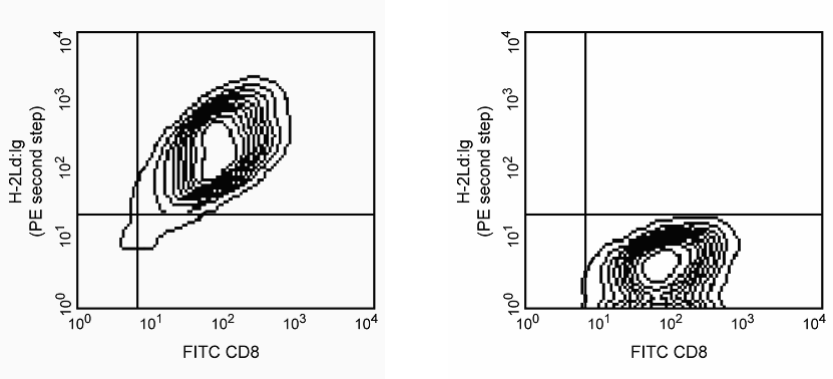
Description

The H-2Ld:Ig fusion protein consists of three extracellular major histocompatibility complex (MHC) class I H-2Ld domains that are fused to the VH regions of mouse IgG1 (see Figure 1). In order for the MHC class I to be functional, i.e., capable of binding peptides, β2 Microglobulin (β2M) must be present. For this reason, the BD Pharmingen DimerX consists of recombinant H-2Ld:Ig fusion protein, supplemented with recombinant β2M. Recombinant MHC molecules, like the DimerX fusion protein, are useful for studying T-cell function by immunofluorescent staining and flow cytometric analysis of antigen-specific T cells.

The MHC gene locus encodes a group of highly polymorphic, cell-surface proteins that play a broad role in the immune response to protein antigens. MHC molecules function by binding and presenting small antigenic protein fragments to antigen-specific receptors expressed by T cells (TCR). Human (human leukocyte antigen/HLA) and mouse (histocompatibility 2/H-2) MHC molecules are structurally and functionally related proteins that comprise two major classes. Class I MHC molecules consist of two separate polypeptide chains. The class I α chain is an MHC encoded, transmembrane polypeptide containing three extracellular domains: α1, α2, and α3. The second chain consists of a non-MHC encoded polypeptide called β2M. Since β2M does not contain a transmembrane domain, it associates with the α chain through noncovalent interaction. Functionally, class I MHC molecules can bind peptides derived from intracellular antigens (e.g., viral and some bacterial antigens) that are specifically recognized by CD8+ T cells. Class II MHC molecules consist of two different transmembrane proteins that can bind peptide fragments derived from extracellular proteins (e.g., bacteria and fungi) and are specifically recognized by CD4+ T cells. TCR recognize both processed peptides bound to MHC, as well as regions of the MHC molecule itself. CD4 and CD8 accessory molecules strengthen formation of the TCR-MHC complex through their interaction with non-polymorphic regions of the MHC molecule.



Schematic representation of the MHC class I:Ig dimeric protein.



Flow cytometric analysis of T cells using DimerX H-2Ld:Ig. *Mouse DimerX I H-2Ld:Ig was incubated with a 40-molar excess of a specific peptide QL9 (QLSPFPFDL, left panel) or an irrelevant peptide MCMV (YPHFMPTNL, right panel) at 4°C for 24 hours. Peptide-loaded H-2Ld:Ig was then used for the immunofluorescent staining of cloned 2C T cells, along with FITC-conjugated anti-mouse CD8a mAb 53-6.7 (Cat. no. 553030/553031) followed by PE-conjugated anti-mouse IgG1 mAb A85-1 (Cat. no. 550083). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C.

The H-2Ld protein was expressed together with human β2M in the mouse plasmacytoma cell line, J558L (ATCC TIB-6). The H-2Ld and β2M polypeptide chains are associated noncovalently as a consequence of their coexpression within J558L cells. The H-2Ld:Ig fusion protein was purified from tissue culture supernatant by affinity chromatography. The purity of the preparation was confirmed by SDS-PAGE.

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Application Notes

Recommended Assay Procedure:

This H-2Ld:Ig fusion protein has been tested by immunofluorescent staining (≤ 4 µg H-2Ld:Ig/million cells) (see Figures) and flow cytometric analysis of antigen specific T cells to assure specificity and reactivity. It is necessary to load the H-2Ld portions of the dimeric protein with a relevant peptide of interest prior to immunofluorescent staining of T cells. H-2Ld:Ig complexes are effectively loaded by incubation with excess relevant (specific) or irrelevant (control) peptides (see Protocol 1). Peptide-loaded H-2Ld:Ig may be used for immunofluorescent staining (see Protocol 2). **Since applications vary, each investigator must determine dilutions appropriate for individual use**.

Protocol 1: Peptide Loading of H-2Ld:Ig Dimeric Protein

An alloreactive T-cell clone, 2C, was originally derived by stimulating BALB/c spleen cells with irradiated P815 (H-2Ld) cells. The specificity of 2C is an endogenous peptide, p2Ca, that is derived from 2-oxoglutarate dehydrogenase, presented by Ld. Several related peptides or peptide analogs have been identified. These differ in their MHC restriction and in their affinity for 2C TCR. The peptides QL9 (QLSPFPFDL) or p2Ca (LSPFPFDL) each have high affinity for H-2Ld.

Several peptide-loading protocols have been described. The method used at BD Biosciences Pharmingen involves passive loading of excess peptide in solution with H-2Ld:Ig protein. We have found that passive loading works particularly well in the case of high-affinity peptides. For lower affinity peptides, an increase in the molar ratio of peptide to H-2Ld:Ig may improve loading, as determined by flow cytometric analysis. It is suggested that for each peptide, parameters such as the dose of H-2Ld:Ig per million cells, molar ratio of peptide to H-2Ld:Ig, and peptide loading time be determined empirically by the investigator. While this DimerX product contains β2 Microglobulin, for investigators requiring excess recombinant Human β2 Microglobulin, we recommend BD Biosciences Cat. No. 551089.

Peptide preparation and loading:

1. The molecular weight (MW) of a peptide of interest will need to be determined. A peptide's MW can be estimated by multiplying its number (n) of amino acids (AA) by 130 daltons (d) per amino acid:

   MW of peptide (d) = n (AA) x 130 (d/AA)

2. A stock of peptide may be prepared at 20 mg/ml in DMSO. Dilute the peptide solution to 2 mg/ml in sterile DPBS, pH 7.2 for use in the H-2Ld:Ig loading protocol.
3. Mix H-2Ld:Ig protein with specific or control peptide at 40, 160, or 640 molar (M) excess.

The following calculation, using an 8 amino acid peptide (8mer) as an example, may be used:

Dp = Molecular Weight of peptide: e.g., 8 amino acids x 130 = 1,040 daltons.

DLd = Molecular Weight of H-2Ld:Ig = 250,000 daltons.

R = desired excess molar ratio, e.g., 160.

Mp = micrograms (µg) peptide of interest.

MLd = micrograms (µg) H-2Ld:Ig in the reaction. A typical amount of peptide-loaded H-2Ld:Ig to use for flow cytometry staining is 0.25 to 4 µg/million cells (test).

$$\mathbf{Mp} = \frac{\text{MLd x R x Dp}}{\text{DLd}} = \frac{\text{4 µg x 160 x 1,040 d}}{\text{250,000 d}} = 2.66\ \mu g$$

Therefore, one would add 2.66 µg of peptide and 4 µg of H-2Ld:Ig in solution for the optimal peptide loading of H-2Ld:Ig.

4. Mix peptide and H-2Ld:Ig together in PBS, pH 7.2, incubate at 37°C overnight. The peptide-loaded H-2Ld:Ig can be stored at 4°C for up to 1 week.

Protocol 2: Immunofluorescent Staining Protocol

1. Prepare peptide-loaded H-2Ld protein staining cocktail by mixing 0.25 - 4 µg of peptide-loaded H-2Ld protein/test with 0.25 - 4 µg 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 µg 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 [e.g., 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 µl. Incubate 10 minutes at 4°C. Add ~1 x 10e6 cells per staining tube (e.g., 12 x 75 mm tube, BD Falcon™ Cat. No. 352008).
4. Add 50 µl 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 [e.g., 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 µl. Incubate 10 minutes at 4°C. Add ~1 x 10e6 cells per staining tube (e.g., 12 x 75 mm tube, BD Falcon™ Cat. No. 352008).

2. Add 0.25 to 4 µg of peptide-loaded H-2Ld: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 µl 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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
550083	PE Rat Anti-Mouse IgG1	0.1 mg	A85-1
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
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/pharmingen/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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