Technical Data Sheet

DimerX I: Recombinant Soluble Dimeric Human HLA-A2:Ig Fusion Protein

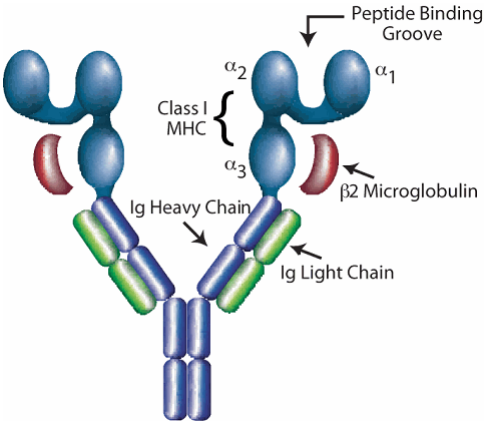
Product Information

Material Number:	**551263**
Size:	0.05 mg
Concentration:	0.5 mg/ml
Isotype:	Mouse IgG1, λ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The HLA-A2:Ig fusion protein consists of three extracellular major histocompatibility complex (MHC) class I HLA-A2 domains that are fused to the VH regions of mouse IgG1 (see schematic representation). 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, BD™ DimerX consists of recombinant HLA-A2:Ig fusion protein, supplemented with recombinant β2M. Recombinant MHC molecules, such as 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.

Preparation and Storage

Store undiluted at 4°C.
The HLA-A2 protein was expressed together with human β2M in the mouse plasmacytoma cell line, J558L (ATCC TIB-6). The HLA-A2 and β2M polypeptide chains are associated noncovalently as a consequence of their coexpression within J558L cells. The HLA-A2: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

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

This HLA-A2:Ig fusion protein has been tested by immunofluorescent staining (≤ 2 µg HLA-A2:Ig/million cells) (see Figure) and flow cytometric analysis of antigen-specific T cells to assure specificity and reactivity. It is necessary to load the HLA-A2 portions of the dimeric protein with a relevant peptide of interest prior to immunofluorescent staining of T cells. HLA-A2:Ig complexes are effectively loaded by incubation with excess relevant (specific) or irrelevant (control) peptides (see Protocol 1). Peptide-loaded HLA-A2:Ig may be used for immunofluorescent staining (see Protocol 2). The FITC-conjugated BB7.2 mAb (anti-human HLA-A2, Cat. No. 551285) is useful for determining the A2 phenotype of cells prior to staining with the HLA-A2:Ig fusion protein. **Since applications vary, each investigator must determine dilutions appropriate for individual use**.

Protocol 1: Peptide Loading of HLA-A2:Ig Dimeric Protein

Several peptide-loading protocols have been described. The method used at BD Biosciences Pharmingen involves passive loading of excess peptide in solution with HLA-A2: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 HLA-A2:Ig may improve loading, as determined by flow cytometric analysis. It is suggested that for each peptide, parameters such as the dose of HLA-A2:Ig per million cells, molar ratio of peptide to HLA-A2:Ig, and peptide loading time be determined empirically by the investigator. Parameters and minimal requirements for peptide binding to HLA-A2 have been reported in the literature. 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 HLA-A2:Ig loading protocol.
3. Mix HLA-A2: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: eg, 8 amino acids x 130 = 1,040 daltons.
DA = Molecular Weight of HLA-A2:Ig = 250,000 daltons.
R = desired excess molar ratio, e.g., 160.
Mp = micrograms (µg) peptide of interest.
MA = micrograms (µg) HLA-A2:Ig in the reaction. A typical amount of peptide-loaded HLA-A2:Ig to use for flow cytometry staining is 1 to 2 µg/million cells (test).

$$\mathbf{Mp} = \frac{MA \times R \times Dp}{DA} = \frac{4\ \mu g \times 160 \times 1{,}040\ d}{250{,}000\ d} = 2.66\ \mu g$$

Therefore, one would add 2.66 µg of peptide and 4 µg of HLA-A2:Ig in solution for the optimal peptide loading of HLA-A2:Ig.

4. Mix peptide and HLA-A2:Ig together in PBS, pH 7.2, incubate at 37°C overnight. The peptide-loaded HLA-A2:Ig can be stored at 4°C for up to 1 week.

Protocol 2: Immunofluorescent Staining Protocol

1. Resuspend PBMC's or target cells in FACS staining buffer [eg, BD Pharmingen™ Stain Buffer with BSA, Cat. No. 554657], at a concentration of approximately 10e6 cells per 50 µl. Add ~1x 10e6 cells per staining tube (eg, 12 x 75 mm tube, BD Falcon™ Cat. No. 352008).
2. Prepare peptide-loaded HLA-A2 protein staining cocktail by mixing 1 - 2 µg of peptide-loaded HLA-A2 protein/test with 1 - 2 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.
3. Add 1 - 2 µg of purified mouse IgG1 isotype control mAb A111-3 (Cat. No. 553485)/test to the staining cocktail (see Step 2 above). Incubate the staining cocktail for 30 minutes at RT, protect from exposure to light.
4. Prepare purified polyclonal human IgG at approximately 0.2 mg/ml in phosphate buffered saline (PBS), pH 7.2.
5. Add 10 µl (2 µg) of human IgG stock per tube to block non-specific binding of DimerX I or antibody reagents to surface Fc receptors. Incubate 10 minutes at RT.
6. Add 50 µl FACS buffer containing the optimal per test amount of the staining cocktail, plus any other cell-surface marker-specific antibodies to be used to each sample.
7. Wash cells 1x with 2 ml FACS buffer, centrifuge for 5 minutes at 250 x g, and aspirate supernatant. Resuspend in FACS buffer and analyze by flow cytometry.*

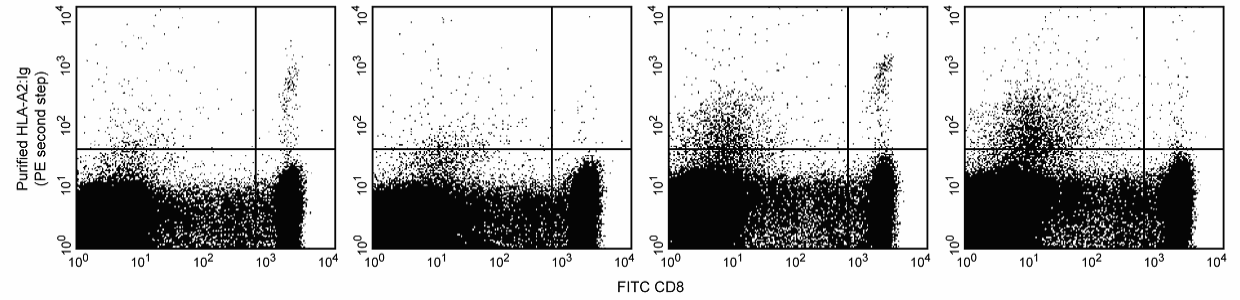
*Within a cell population, the frequency of cells which are capable of recognizing specific peptide-MHC complexes is typically very low, eg, <1%. We recommend acquisition of at least 100,000 lymphocytes for flow cytometric analysis for optimal detection of this subpopulation.

Protocol 3: Alternative: Immunofluorescent Staining Protocol

1. Resuspend PBMC's or target cells in FACS staining buffer [e.g., BD Pharmingen™ Stain Buffer with BSA, Cat. No. 554657], at a concentration of approximately 10e6 cells per 50 µl. Add ~1x 10e6 cells per staining tube (eg, 12 x 75 mm tube, BD Falcon™ Cat. No. 352008).
2. Prepare purified polyclonal human IgG at approximately 0.2 mg/ml in phosphate buffered saline (PBS), pH 7.2.
3. Add 10 µl (2 µg) of human IgG stock per tube to block non-specific binding of DimerX I or antibody reagents to surface Fc receptors. Incubate 10 minutes at RT.
4. Add 1 to 2 µg of peptide-loaded HLA-A2:Ig protein to each sample. Incubate 60 minutes at 4°C.
5. Wash cells 1x with 2 ml FACS buffer, centrifuge for 5 minutes at 250 x g, and aspirate supernatant.
6. Again add 10 µl (2 µg) of purified polyclonal human IgG per sample. Incubate 10 minutes at RT.
7. Add 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 minutes at RT.**
8. Wash cells 1x with 2 ml FACS buffer, centrifuge for 5 minutes at 250 x g, and aspirate supernatant. Resuspend in FACS buffer and analyze by flow cytometry.***

Additional antibodies specific for markers such as CD4, CD8, or HLA-A2 may be included at this step by adding appropriately diluted fluorescently-conjugated antibodies. **NOTE: BD™ Dimerx HLA-A2:Ig is a fusion protein containing mouse IgG1 heavy-chain regions. Therefore, it is important to choose reagents that are of a different isotype to avoid possible staining by the secondary reagent (anti-IgG1) with these other antibody reagents.

***Within a cell population, the frequency of cells which are capable of recognizing specific peptide-MHC complexes is typically very low, eg, <1%. We recommend acquisition of at least 100,000 lymphocytes for flow cytometric analysis for optimal detection of this subpopulation.



Comparison of BD™ DimerX HLA-A2:Ig staining of normal human lymphocytes from a cytomegalovirus (CMV) seropositive donor using two different protocols. *PBMCs from an HLA-A2+ CMV-infected donor were stained with FITC anti-human CD8 (clone G42-8), Cat. No. 551347) and with purified HLA-A2:Ig dimer loaded with a 640-molar excess of CMV pp65-derived (NLVPMVATV), HLA-A2-binding peptide (far left panel), or unloaded purified HLA-A2:Ig dimer (middle left panel) after preincubation of the dimer with PE anti-mouse IgG1 (clone A85-1, Cat. No. 550083) as described in Protocol 2 on this document. Alternatively, PBMCs were stained with FITC anti-human CD8 and purified HLA-A2:Ig dimer loaded with CMV peptide (middle right panel), or unloaded purified HLA-A2:Ig dimer (far right panel) and then with PE anti-mouse IgG1 as described in Protocol 3 on this document. Antibody conjugates were chosen to be non-IgG1 isotypes so as not to interfere with detection of HLA-A2:Ig staining. Cells were analyzed using a lymphocyte gate (gate not shown). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Suggested Companion Products

Catalog Number	Name	Size	Clone
551285	FITC Mouse Anti-Human HLA-A2	0.1 mg	BB7.2
551089	Recombinant Human β2 Microglobulin	0.1 mg	(none)
550083	PE Rat Anti-Mouse IgG1	0.1 mg	A85-1
553485	Purified Mouse IgG1 λ Isotype Control	0.5 mg	A111-3
551347	FITC Mouse Anti-Human CD8	0.1 mg	G42-8
554657	Stain Buffer (BSA)	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

Dal Porto J, Johansen TE, Catipovic B, et al. A soluble divalent class I major histocompatibility complex molecule inhibits alloreactive T cells at nanomolar concentrations. *Proc Natl Acad Sci U S A.* 1993; 90(14):6671-6675.(Biology)

Khilko SN, Jelonek MT, Corr M, Boyd LF, Bothwell AL, Margulies DH. Measuring interactions of MHC class I molecules using surface plasmon resonance. *J Immunol Methods.* 1995; 183(1):77-94.(Biology)

Parker KC, Bednarek MA, Hull LK, et al. Sequence motifs important for peptide binding to the human MHC class I molecule, HLA-A2. *J Immunol.* 1992; 149(11):3580-3587.(Biology)

Parker KC, Carreno BM, Sestak L, Utz U, Biddison WE, Coligan JE. Peptide binding to HLA-A2 and HLA-B27 isolated from Escherichia coli. Reconstitution of HLA-A2 and HLA-B27 heavy chain/beta 2-microglobulin complexes requires specific peptides. *J Biol Chem.* 1992; 267(8):5451-5459.(Biology)

Schneck JP, Slansky JE, O'Herrin SM, Greten TF. Monitoring antigen-specific T cells using MHC-Ig dimers. In: Coligan J, Kruisbeek D, Margulies EM, Shevach EM, Strober W, ed. *Current Protocols in Immunology*. New York: John Wiley & Sons, Inc; 2000:17.2.1-17.2.17.(Methodology)