DimerX I: Recombinant Soluble Dimeric Mouse H-2K[b]:Ig Fusion Protein

Product Information

Material Number: 550750
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-2K[b]:Ig fusion protein consists of three extracellular major histocompatibility complex (MHC) class I H-2Kb domains that are fused to the VH regions of mouse IgG1. 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 H-2K[b]:Ig fusion protein, supplemented with recombinant β2M. Recombinant MHC molecules, like the BD 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 nonpolymorphic regions of the MHC molecule.

Preparation and Storage

Store undiluted at 4°C.

The H-2K[b] protein was expressed together with human β2M in the mouse plasmacytoma cell line, J558L (ATCC TIB-6). The H-2K[b] and β2 polypeptide chains are associated noncovalently as a consequence of their coexpression within J558L cells. The H-2K[b]: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-2K[b]:Ig fusion protein has been tested by immunofluorescent staining (≤ 4 µg H-2K[b]: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 H-2K[b] portions of the dimeric protein with a relevant peptide of interest prior to immunofluorescent staining of T cells. H-2K[b]:Ig complexes are effectively loaded by incubation with excess relevant (specific) or irrelevant (control) peptides (see Protocol 1). Peptide-loaded H-2K[b]: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-2K[b]:Ig Dimeric Protein
An alloreactive T-cell clone, 2C, is specific for an endogenous peptide, p2Ca. Several related peptides or peptide analogs have been identified. These differ in their MHC restriction and in their affinity for 2C TCR. For H-2Kb, SIY peptide can also be recognized by 2C TCR in the context of H-2K[b]. SIY has a relatively high affinity for H-2K[b] and is suggested as a positive control for staining of 2C cells in this assay. The 2C clone was originally derived by stimulating BALB/c spleen cells with irradiated P815 (H-2Ld) cells.

Several peptide-loading protocols have been described. The method used at BD Biosciences Pharmingen involves passive loading of excess peptide in solution with H-2K[b]:Ig protein. We have found that this 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-2K[b]:Ig may improve loading, as determined by flow cytometric analysis. It is suggested that for each peptide, parameters such as the dose of H-2K[b]:Ig per million cells, molar ratio of peptide to H-2K[b]:Ig, and peptide loading time be determined empirically by the investigator. While this BD 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:
   \[ \text{MW of peptide (d)} = n \times 130 \text{ d/AA} \]
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-2K[b]:Ig loading protocol.
3. Mix H-2K[b]: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:
   \[
   \begin{align*}
   D_p &= \text{Molecular Weight of peptide: e.g., 8 amino acids x 130 = 1,040 daltons.} \\
   D_K[b] &= \text{Molecular Weight of H-2K[b] : Ig = 250,000 daltons.} \\
   R &= \text{desired excess molar ratio, e.g., 160.} \\
   M_p &= \text{micrograms (µg) peptide of interest.} \\
   M_K[b] &= \text{micrograms (µg) H-2K[b]:Ig in the reaction. A typical amount of peptide-loaded H-2K[b]:Ig to use for flow cytometry staining is 0.25 to 4 µg/million cells (test).} \\
   M_p &= \frac{M_K[b] \times R \times D_p}{D_K[b]} = \frac{4 \times 160 \times 1,040}{250,000} = 2.66 \text{ µg}
   \end{align*}
   \]
   Therefore, one would add 2.66 µg of peptide and 4 µg of H-2K[b]:Ig in solution for the optimal peptide loading of H-2K[b]:Ig.

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

Protocol 2: Immunofluorescent Staining Protocol

1. Prepare peptide-loaded H-2K[b] protein staining cocktail by mixing 0.25-4 µg of peptide-loaded H-2K[b] protein/test with 0.25-4 µg of PE-conjugated A85-1 mAb (anti-mouse IgG1, Cat. No. 550083)/test. Incubate the staining cocktail for 30 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 BD 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. Mix peptide and H-2K[b]:Ig together in PBS, pH 7.2, incubate at 37°C overnight. The peptide-loaded H-2K[b]:Ig can be stored at 4°C for up to 1 week.

Protocol 3: Alternative: Immunofluorescent Staining Protocol
1. Resuspend mouse cells in BD 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 - 4 µg of peptide-loaded H-2K[b]:Ig protein to cell suspension. Incubate 60 minutes at 4°C.
3. Wash cells 1x with 2 ml BD FACS buffer, centrifuge for 5 minutes at 250 µg, and aspirate supernatant.
4. Resuspend cells in 100 µl BD 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 BD 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**

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<th>Name</th>
<th>Size</th>
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<td>PE Rat Anti-Mouse IgG1</td>
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<td>A85-1</td>
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<tr>
<td>553485</td>
<td>Purified Mouse IgG1 λ Isotype Control</td>
<td>0.5 mg</td>
<td>A111-3</td>
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<tr>
<td>551089</td>
<td>Recombinant Human β2 Microglobulin</td>
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<td>Stain Buffer (FBS)</td>
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<td>553141</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
<td>0.1 mg</td>
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**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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**


