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Product datasheet for SM3053A

HLAE (HLA-E) Mouse Monoclonal Antibody [Clone ID: MEM-E/02]

Product data:

Product Type:	Primary Antibodies
Clone Name:	MEM-E/02
Applications:	WB
Recommended Dilution:	**Western blotting:** 1-5 µg/mL for chemiluminescence detection system. Detailed procedure is provided in**Protocols.**
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant Human HLA-E.
Specificity:	This antibody reacts with the HLA-E denatured heavy chain (43 kDa) on Western blotting but does not recognize native HLA-E molecule.
Formulation:	PBS, pH 7.2 containing 50% Glycerol without preservatives. State: Azide Free State: Liquid purified IgG fraction.
Concentration:	lot specific
Purification:	Protein-A Sepharose Chromatography of hybridoma supernatant.
Conjugation:	Unconjugated
Storage:	Store the antibody (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	major histocompatibility complex, class I, E
Database Link:	Entrez Gene 3133 Human P13747

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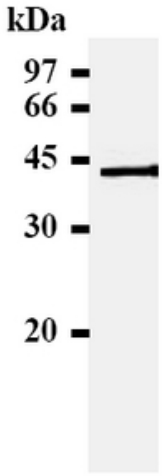
Background: HLA-E (human leucocyte antigen-□E) is a conserved class I major histocompatibility molecule. It binds to the leader peptide derived from the polymorphic classical MHC molecules HLA-A, HLA-B and HLA-C. This peptide binding stabilizes the HLA-E protein and allows it to migrate to the cell surface. HLA-E then interacts with CD94/NKG2A receptors on natural killer cells. This interaction inhibits natural killer cell-mediated lysis of cells displaying HLA-E. In virally infected or tumor cells, down-regulation of HLA-A, HLA-B and HLA-C production prevents stabilization of HLA-E by the leader peptide. Under these circumstances, HLA-E is degraded before it reaches the cell surface and the cell is then vulnerable to lysis by natural killer cells.

Synonyms: HLA-6.2, HLAE, MHC class I antigen E

Note: This product was originally produced by MBL International.

Protocol: **SDS-PAGE & Western Blotting**
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10%glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at RT.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.
Positive Controls: Human Placenta

Product images:



Western blot anaylsis: SM3053A HLA-E antibody staining of Human Placenta extracts