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Product datasheet for AM26593AF-N

Hsp40 (DNAJB1) (1-70) Mouse Monoclonal Antibody [Clone ID: 2E1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	2E1
Applications:	IP, WB
Recommended Dilution:	**Western blot:** 1-2 µg/ml for chemiluminescence detection system. **Immunoprecipitation:** 2-5 µg/200 µl of cell extract from 10^6 cells. For details see protocols below.
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Full-length human HSP40 (1-340 aa)
Specificity:	This antibody recognizes J domain (1-70 aa) of hsp40.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	DnaJ heat shock protein family (Hsp40) member B1
Database Link:	Entrez Gene 3337 Human P25685

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Background: 40 kDa heat shock protein (hsp40) is a novel heat shock protein induced by heat shock and other stresses in mammalian and avian cells. The cDNA sequence of the hsp40 is id entical with HDJ1. Deduced amino acid sequence of the hsp40 cDNA is homologues to DnaJ, an E. coli heat shock protein and its homologues in yeast such as SCJ1, YDJ1 (MAS5), SIS1, SEC63 and zuotin. E coli 's DnaJ heat shock protein is known to function together with DnaK (hsp70) and GrpE as a molecular chaperone, which is necessary for assembly and disassembly of protein complexes, protein folding, renaturation of denatured proteins, prevention of protein aggregation and protein export. The hsp40 is colocarized with hsp70 in the nuclei and nucleoli of heat-shocked HeLa cells which suggests that hsp70 (DnaK)-hsp40 (DnaJ) chaperone system is ubiquitous.

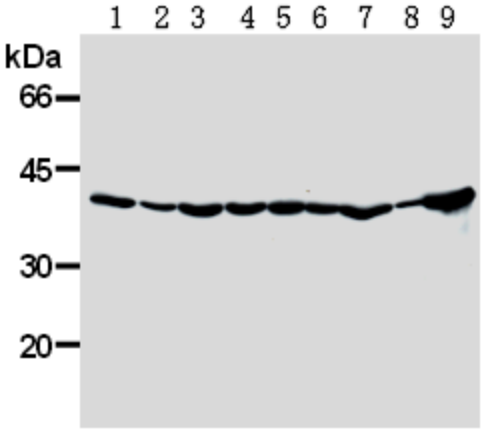
Synonyms: Heat shock 40 kDa protein 1, HSP-40, DnaJ protein homolog 1, HDJ-1, DNAJ1, HDJ1, HSPF1

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 o 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 o 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 µ L 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/cm 2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufact ure'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 o 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 room temperature.
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 for Western blotting; Jurkat, Raji, HeLa, MRC-5, ZR-75-1, NIH/3T3, WR19L, P19, PC12)
Immunoprecipitation
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 o 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 o C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the APPLICATIONS into 300 µ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4 o C. Add 20 µ L of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4 o C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µ L/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.

Product images:



Western blot analysis of hsp40 expression in Jurkat (1), Raji (2), HeLa (3), MRC-5 (4), ZR-75-1 (5), NIH/3T3 (6), WR19L (7), P19 (8) and PC12 (9) using AM26593AF-N.