

MONOCLONAL ANTIBODY

Anti-Hsp40 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M039-3	2E1	Mouse IgG2a	100 µL	1 mg/mL

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 identical with HDJ1. Deduced amino acid sequence of the Hsp40 cDNA is homologous 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 colocalized with Hsp70 in the nuclei and nucleoli of heat-shocked HeLa cells which suggests that Hsp70 (DnaK)-Hsp40 (DnaJ) chaperone system is ubiquitous.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 2E1) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the full-length human Hsp40 (1-340 aa).

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody recognizes J domain (1-70 aa) of human Hsp40 and reacts with Hsp40 on Western blotting and Immunoprecipitation (40 kDa).

APPLICATIONS:

- Western blotting; 1-2 µg/mL
- Immunoprecipitation; 2-5 µg/200 µL of cell extract from 5 x 10⁶ cells
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested
- Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, U937, Raji, HeLa, HL-60, ZR-75-1, MRC-5	NIH/3T3, P19, Ba/F3, WR19L	PC12
Reactivity on WB	+	+	+

INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

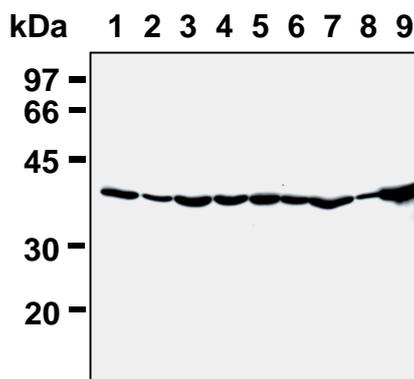
REFERENCES:

- 1) Helmlinger, D., *et al.*, *Hum. Mol. Genet.* **11**, 3351-3359 (2002)
- 2) Sugito, K., *et al.*, *FEBS Letter* **358**, 161-164 (1995)
- 3) Frydman, J., *et al.*, *Nature* **370**, 111-117 (1994)
- 4) Ohtsuka, K. *et al.*, *Exp. Cell Res.* **209**, 357-366 (1993)
- 5) Hattori, H., *et al.*, *J. of Cell Sci.* **104**, 629-638 (1993)
- 6) Ohtsuka, K., *Biochem. Biophys. Res. Commun.* **197**, 235-240 (1993)
- 7) Hattori, H., *et al.*, *Cell Struct. Funct.* **17**, 77-86 (1992)
- 8) Ohtsuka, K., *et al.*, *Biochem. Biophys. Res. Commun.* **166**, 642-647 (1990)

Clone 2E1 is used in reference number 1).

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Western blotting 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 M039-3.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western Blotting

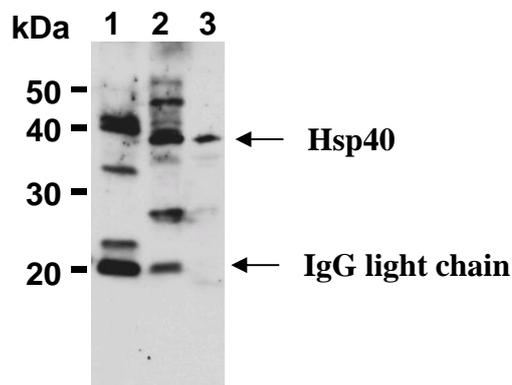
- 1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) 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 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/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
- 9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL, code no. 330) 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).
- 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 1 minute.
- 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 volumes 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.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 300 μ L of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
- 4) 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°C.
- 5) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 6) Resuspend the agarose with cold IP buffer [10 mM Tris-HCl (pH 7.4), 250 mM NaCl, 0.1% NP-40].
- 7) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 8) Repeat steps 6)-7) 2-4 times.
- 9) Resuspend the beads in 30 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 μ L/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting**.)



Immunoprecipitation of Hsp40 from Raji with mouse IgG2a (1) or M039-3 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M039-3. Raji crude lysate was resolved in lane 3.