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

POLYCLONAL ANTIBODY

Anti-HSP 27

Code No.	Quantity	Form
PD001	100 µL	Affinity Purified

BACKGROUND: The 27 kDa of Heat shock protein (Hsp27) is expressed in a variety of cells including breast and ovarian cancer, muscle, skin, motor and sensory neurons. Its expression has been associated with the presence of estrogen receptors, thermotolerance, lower cell proliferation, drug resistance, maintenance of myofibrillar integrity, and protection against necrotic and apoptotic cell death. It is rapidly phosphorylated in cells following a variety of stresses, which causes a decrease in oligomer size. In Hsp25 and Hsp27, this phosphorylation is the result of an activated p38 MAP kinase cascade.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with HSP27 purified from human muscle.

FORMULATION: 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 reacts with human HSP27 on Western blotting.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, U937, ZR-75-1, MCF7, HPB-ALL	NIH/3T3, WR19L	Rat-1, PC12	BHK
Reactivity on WB	+	-	-	-

INTENDED USE:

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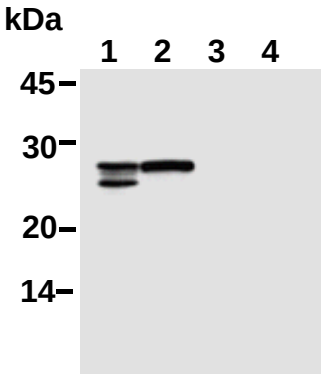
REFERENCE:

1) Inaguma, Y., *et al.*, *J. Biochem.* **114**, 378-384 (1993)

RELATED PRODUCT:

M039-3 Anti-HSP40 (2E1)



Western blot analysis of HSP27 expression in U937 (1), HeLa (2), WR19L (3) and PC12 (4) using PD001.

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/cm^2 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-rabbit IgG (MBL; code no. 458) 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; HeLa, U937, ZR-75-1, MCF7, HPB-ALL)