NEUROMICS

HSP60 (D85) Data Sheet

Catalog Number:	RA18030	**Host:**	Rabbit
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human, Mouse, Rat and Primate
Immunogen Sequence:	Synthetic peptide (KLH-coupled) surrounding Asp85 of human HSP60.	**Format:**	Liquid in 10mM sodium HEPES (pH7.5), 150mM NaCl, 100ug BSA and 50% glycerol.

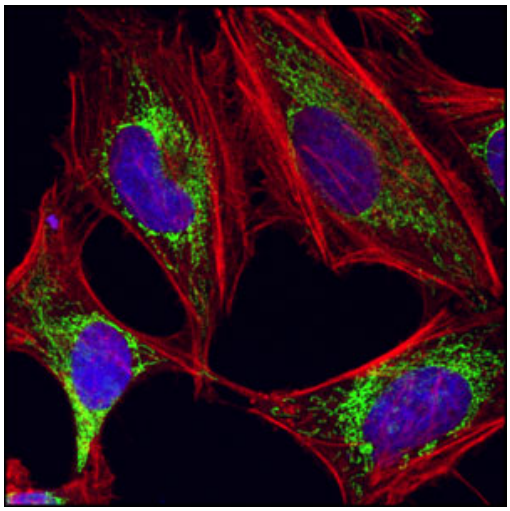
Applications: Immunohistochemistry 1:100
Western Blot 1:1000
Flow Cytometry: 1:50
Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store at -20ºC. *Do not aliquot the antibody*. Stable for at least 6 months. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

Application Notes

Description/Data:
Hsp60 is a member of a highly conserved family which includes molecular chaperones. Consistent with their functions as chaperones, Hsp60 and Hsp10 have been suggested to act as docking molecules with a passive role in the maturation of caspase processing, and are primarily known as a mitochondrial protein that is important for folding key proteins after import into the mitochondria . It is now clear that a significant amount of HSP60 is also present in the cytosol of many cells and that it is induced by stress, inflammatory and immune responses, autoantibodies correlated with Alzheimer's, coronary artery diseases, MS, and diabetes.

Image: Confocal immunofluorescent analysis of HeLa cells using HSP60 (D85) Antibody (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5™ (fluorescent DNA dye).



Specificity:
HSP60 (D85) Antibody detects endogenous levels of total HSP60 protein. This antibody does not cross-react with other HSPs.

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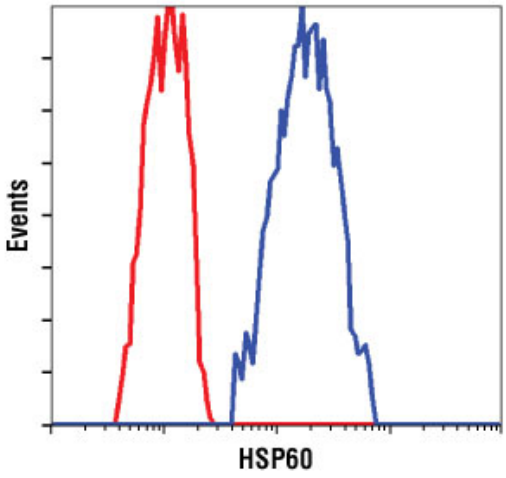

Image: Flow cytometric analysis of HeLa cells using HSP60 (D85) Antibody (blue) compared to a nonspecific negative control antibody (red).

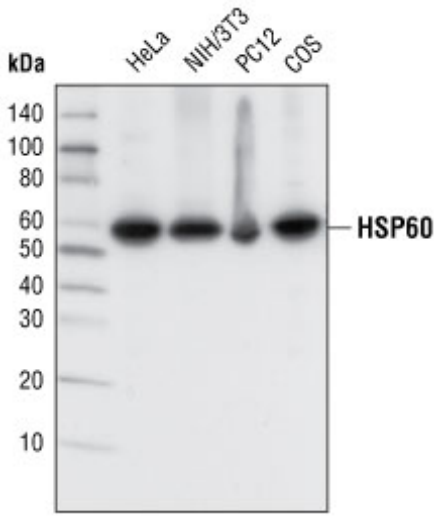

Image: Western blot analysis of extracts from various cell types using HSP60 (D85) Antibody.

Western Blot:
Sample Preparation
1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape cells from plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose (or PVDF) membrane.
Membrane Blocking and Antibody Incubations
Note: Volumes for 10 cm x 10 cm (100 cm2) membrane; for different sized membranes, adjust vol. accordingly.
1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.
8. Process membranes using enhanced chemiluminescence.

Immunohistochemistry:
NOTE: All subsequent incubations should be carried out at room temperature, unless otherwise noted, in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.
1. Block specimen in 5% normal serum from same species as secondary antibody (eg. normal goat serum, normal donkey serum) in PBS/Triton for 60 minutes.
2. While blocking, prepare primary antibody by diluting as indicated on datasheet in PBS/Triton. You will need 50-100 µl per section, 25-50 µl per coverslip, chamber, or well (48 or 96 well plate).
3. Aspirate blocking solution, apply diluted primary antibody.
NOTE: For double-labeling, prepare a cocktail of mouse and rabbit primary antibodies at their appropriate dilutions in PBS/Triton.
4. Incubate overnight at 4°C.

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5. Rinse three times in PBS for 5 minutes each.
OPTION: To decrease background stain, rinse in high salt PBS for two minutes between second and third PBS rinses. Be aware, this may reduce specific staining of some antibodies.
NOTE: If using primary antibodies directly conjugated with CHROMEO™sity Secondary Antibodies then skip to step C8.
6. Incubate in fluorochrome-conjugated secondary antibody diluted in PBS/Triton for 1-2 hours at room temperature in dark.
NOTE: For double-labeling, prepare a cocktail of fluorochrome-conjugated anti-mouse and anti-rabbit primary antibodies at their appropriate dilutions in PBS/Triton.
7. Rinse in PBS/high salt PBS as in step 5.
8. Coverslip slides with Vectashield Mounting Medium or apply just enough to cover cells in multiwell plate.
9. Seal slides by painting around edges of coverslips with nail polish.
10. Examine specimens immediately using appropriate excitation wavelength, depending on fluorochrome for best results or store flat at 4°C in dark.

Flow-Cytometry
A Solutions and Reagents
1. 1X Phosphate Buffered Saline (PBS): Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na2HPO4 and 0.24 g KH2PO4 in 800 mL distilled water (dH2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. Incubation Buffer: Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

B Fixation
1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization
1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at –20°C in 90% methanol.

D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies
NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.
1. Aliquot 0.5-1x106 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100 µl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
6. Incubate for 30-60 minutes at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in fluorochrome-conjugated secondary antibody*, diluted in Incubation Buffer according to the manufacturer's recommendations.
9. Incubate for 30 minutes at room temperature.
10. Rinse as before in Incubation Buffer by centrifugation.
11. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.
*Recommended Secondary Antibody: CHROMEO™sity 488 Goat-anti-Rabbit IgG (Catalog#: GT26001).

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