

Affinity-Purified Goat Anti-human Heat Shock Protein 90 (HSP90) Antibody

ORDERING INFORMATION

Catalog Number: AF3286

Lot Number: WLU01

Size: 100 µg

Storage: -20° C

Specificity: human HSP90 Alpha and Beta

Immunogen: *E. coli* derived recombinant human HSP90β (aa 1-724)

Ig Type: goat IgG

Application: Western blot

Background

The heat shock protein-90 kDa (HSP90) is a composite name for a large group of genes whose molecular weights average 90 kDa. HSP90 functions primarily as a molecular chaperone, facilitating the folding of other cellular proteins, preventing protein aggregation, or targeting improperly folded proteins to specific degradative pathways. HSP90 is ubiquitously expressed, highly conserved and accounts for 1-2% of the total cellular protein. Recently introduced, standardized nomenclature has divided the 17 identified HSP90 genes into three related and one unrelated classes, HSP90aa, HSP90AB, HSP90BB, and TRAP, respectively. Six of these genes were functional while the remaining 11 are considered putative pseudogenes. Eukaryotic cells have two principal isoforms of HSP90. The antibody described here is to HSP90AB1-isoform 1, a 724 amino acid protein that is constitutively expressed. HSP90AB1-1 is also known as HSP90Beta, HSP90B, HSPCB, HSPC2, and HSP89-Beta. The inducible form, HSP90AA1, is a 737 amino acid protein also known as HSP90-Alpha, HSP90A, HSPC1, HSPCA, HSP89-Alpha and LAP2. HSP90AB1-1 and HSP90AA are share 90% identity. In addition to its role as a molecular chaperone and stress response protein, HSP90 is a central component in a number of basic cellular processes including hormone signaling and cell cycle control.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human Heat Shock Protein 90 Beta (rhHSP90β; amino acids 1 - 724; GenBank Accession # NM_007355). Polyclonal antibody was affinity-purified on a column derivatized with recombinant human HSP90β.

Formulation

Lyophilized from a 0.2 µm-filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute the antibody in 100 µL PBS containing 0.02% NaN₃. The antibody concentration will be 1.0 mg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquoted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

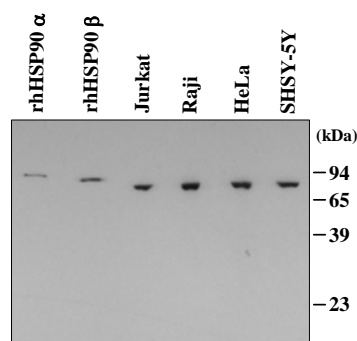
Specificity

The antibody is known to react with endogenous human HSP90 on Western blots. Goat anti-human HSP-90 detects HSP90α and HSP90β.

Applications

Western blot - An antibody concentration of 0.5 µg/mL is recommended.

Optimal dilutions should be determined by each laboratory for each application.



Detection of HSP90 with AF3286.

Recombinant human HSP90α, HSP90β (5 ng/lane) and lysates from human Jurkat, Raji, HeLa, and SHSY-5Y cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 0.5 µg/mL goat anti-HSP90 as described in Protocols for Immunoblotting. A one minute exposure to film is shown.

Protocols for Immunoblotting:

Western blotting

<u>Blotting Buffer</u>	<u>Blocking Solution</u>	<u>Antibody Solution</u>
25 mM Tris, pH 7.4	5% nonfat dry milk in Blotting Buffer	2% nonfat dry milk in Blotting Buffer
0.15 M NaCl		
0.1% Tween [®] 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in antibody solution containing 0.5 µg/mL goat anti-human HSP90.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane for 1 hour at room temperature in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detect with Western Glo Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

Cell lysates for Western blots: To prepare total cell lysates, cells are solubilized in hot 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2×10^6 - 1×10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with 3 - 4 bursts of 5-10 second each. Samples are diluted with 1X SDS sample buffer to the desired concentration.

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