

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat gp96/HSP90B1 in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human gp96/HSP90B1 Arg503-Arg660 Accession # P14625
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

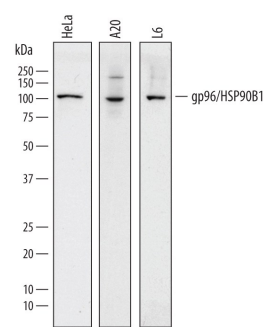
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunohistochemistry	1-15 µg/mL	See Below
Knockout Validated	Gp96/HSP90B1 is specifically detected in HEK293T human embryonic kidney parental cell line but is not detectable in gp96/HSP90B1 knockout HEK293T cell line.	

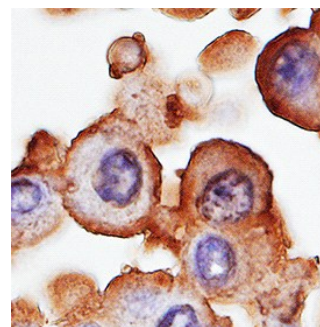
DATA

Western Blot



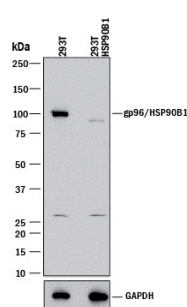
Detection of Human, Mouse, and Rat gp96/HSP90B1 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, A20 mouse B cell lymphoma cell line, and L6 rat myoblast cell line. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human/Mouse/Rat gp96/HSP90B1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7606) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for gp96/HSP90B1 at approximately 100 kDa (as indicated). This experiment was conducted under reducing conditions and using *Immunoblot Buffer Group 1*.

Immunohistochemistry



gp96/HSP90B1 in Human Mesothelioma Tissue. gp96/HSP90B1 was detected in immersion fixed paraffin-embedded sections of human mesothelioma tissue using Sheep Anti-Human/Mouse/Rat gp96/HSP90B1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7606) at 1 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and plasma membrane. View our protocol for *Chromogenic IHC Staining of Paraffin-embedded Tissue Sections*.

Knockout Validated



Western Blot Shows Human gp96/HSP90B1 Specificity by Using Knockout Cell Line. Western blot shows lysates of HEK293T human embryonic kidney parental cell line and gp96/HSP90B1 knockout HEK293T cell line (KO). PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human/Mouse/Rat gp96/HSP90B1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7606) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for gp96/HSP90B1 at approximately 100 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in knockout HEK293T cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using *Immunoblot Buffer Group 1*.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none">● 12 months from date of receipt, -20 to -70 °C as supplied.● 1 month, 2 to 8 °C under sterile conditions after reconstitution.● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Glycoprotein 96 (gp96; also endoplasmic, GRP-94, TRA1 and HSP90B1) is a 94-100 kDa member of the HSP 90 family of proteins. Gp96 is a ubiquitously-expressed, ER resident protein, and is found in a preformed complex with BiP, CaBP1 and UDP-glucosyltransferase. This is a chaperone complex that binds unfolded protein substrates. When folded properly, the substrate is forwarded to calnexin-containing chaperone complexes that promote its maturation. Gp96 clients are restricted, and include disulfide-bonded integrins, TLRs, LDLR and CD180. Within the complex, gp96 exists as a disulfide-linked homodimer that may form higher-order oligomers. Gp96 also appears on the cell surface, and may serve as a receptor for bacteria. Mature human gp96 is a 782 amino acid (aa) membrane-associated protein (aa 22-803). It is not a transmembrane protein, but utilizes an ER retention signal (aa 800-803) to interact with the ER membrane. The molecule possesses a HATPase-C like region (aa 98-219), plus multiple ATP binding and two utilized phosphorylation sites. Over aa 503-660, human gp96 shares 98% aa sequence identity with mouse gp96.