Anti-GRP94 Antibody

ER1511-5

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 92 kDa

Description: Heat shock protein (HSP) molecular chaperones are environmental stress-inducible gene products. The human HSP 90 family includes 17 genes that fall into four classes: HSP90AA, HSP90AB, HSP90B and TRAP. HSP 90 family members guide the normal folding, intracellular disposition and proteolytic turnover of many key regulators of cell growth, differentiation and survival. HSP 90α, also designated HSP90A, HSP 86 and LPS-associated protein 2 (LAP2), is a cytosolic enhancer of inducible nitric-oxide synthase (iNOS), with chaperone activity that is important for the transcriptional activity of p53. HSP 90β, also designated HSP90B, HSP 84 and HSPC2, is a cytosolic protein that participates in signaling pathways with PKC e to protect cells from external damage, particularly in heat shock-mediated events. GRP 94, also known as tumor rejection antigen 1 (TRA1), ECGP and GP96, localizes to the ER, is highly expressed in BGC-823 human gastric carcinoma cells and is upregulated in human endothelial cells in response to hypoxia by HIF-1. TRAP1 (TNF receptor-associated protein 1), also designated HSP 75) is a mitochondrial matrix component that plays a role in the induction of apoptosis in response to reactive oxygen species.

Immunogen: Synthetic peptide within human GRP94 aa 194-247.

Positive control: HepG2 cell lysate, HeLa cell lysate, human brain tissue lysate, NIH/3T3 cell lysate, mouse placenta tissue lysate, mouse brain tissue lysate, PC-12 cell lysate, rat placenta tissue lysate, rat brain tissue lysate, HeLa, NIH/3T3, PC-12 , human liver tissue, human breast tissue, human stomach cancer tissue, mouse stomach tissue.

Subcellular location: Endoplasmic reticulum lumen, Melanosome.

Database links: SwissProt: P14625 Human | P08113 Mouse | Q66HD0 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:500
IHC-P	1:100-1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Immunogen affinity purified.

Images

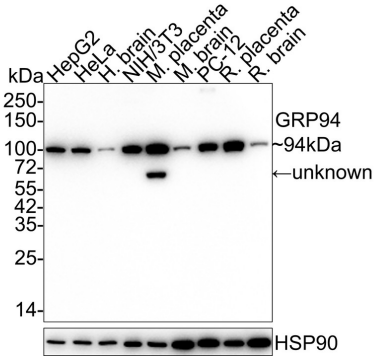

Fig1: Western blot analysis of GRP94 on different lysates with Rabbit anti-GRP94 antibody (ER1511-5) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate (10 µg/Lane)
Lane 2: HeLa cell lysate (10 µg/Lane)
Lane 3: Human brain tissue lysate (20 µg/Lane)
Lane 4: NIH/3T3 cell lysate (10 µg/Lane)
Lane 5: Mouse placenta tissue lysate (20 µg/Lane)
Lane 6: Mouse brain tissue lysate (20 µg/Lane)
Lane 7: PC-12 cell lysate (10 µg/Lane)
Lane 8: Rat placenta tissue lysate (20 µg/Lane)
Lane 9: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 92 kDa
Observed band size: 94 kDa

Exposure time: 25 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ER1511-5) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

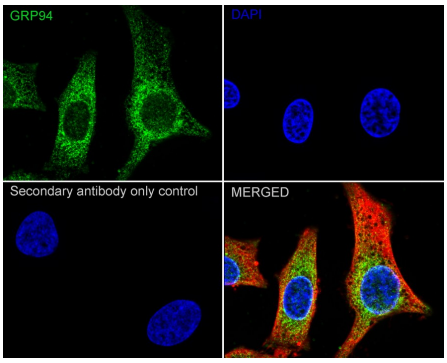

Fig2: Immunocytochemistry analysis of HeLa cells labeling GRP94 with Rabbit anti-GRP94 antibody (ER1511-5) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GRP94 antibody (ER1511-5) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

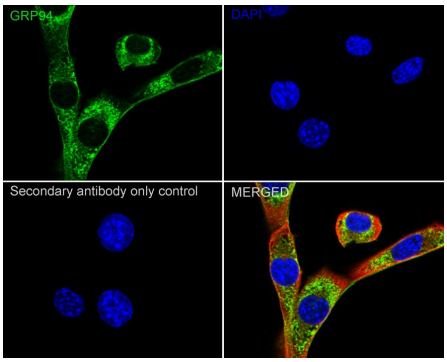

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling GRP94 with Rabbit anti-GRP94 antibody (ER1511-5) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GRP94 antibody (ER1511-5) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

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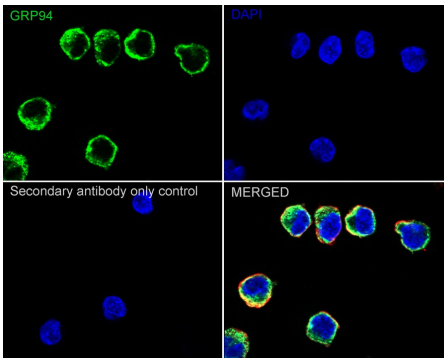

Fig4: Immunocytochemistry analysis of PC-12 cells labeling GRP94 with Rabbit anti-GRP94 antibody (ER1511-5) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GRP94 antibody (ER1511-5) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

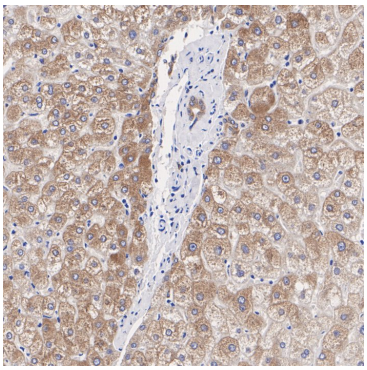

Fig5: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-GRP94 antibody (ER1511-5) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1511-5) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

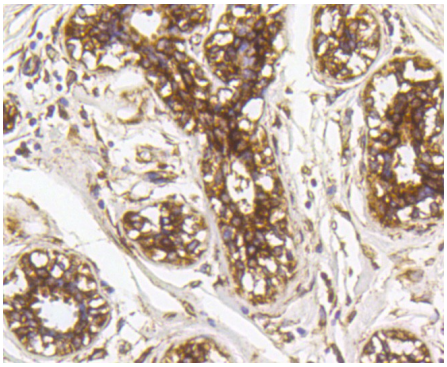

Fig6: Immunohistochemical analysis of paraffin-embedded human breast tissue using anti-GRP94 antibody. Counter stained with hematoxylin.

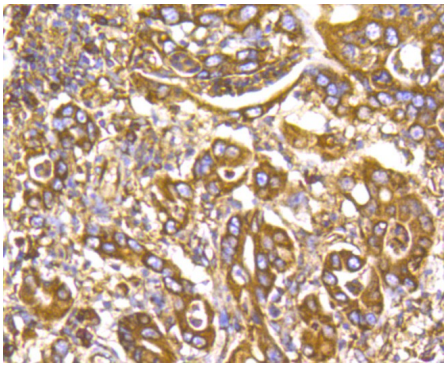

Fig7: Immunohistochemical analysis of paraffin-embedded human stomach cancer tissue using anti-GRP94 antibody. Counter stained with hematoxylin.

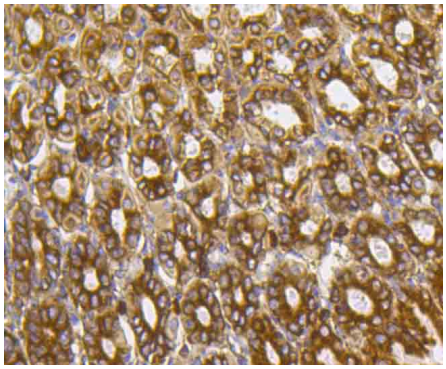

Fig8: Immunohistochemical analysis of paraffin-embedded mouse stomach tissue using anti-GRP94 antibody. Counter stained with hematoxylin.

Background References

1. Gallagher CM et al. Ceapins are a new class of unfolded protein response inhibitors, selectively targeting the ATF6a branch. Elife 5.pii: e11878 (2016).
2. Plate L et al. Small molecule proteostasis regulators that reprogram the ER to reduce extracellular protein aggregation. Elife 5.pii: e15550 (2016).