Anti-GRP78 / BIP Antibody [C9-9-R]

HA601076

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 72 kDa
Clone number:	C9-9-R

Description: Endoplasmic reticulum chaperone that plays a key role in protein folding and quality control in the endoplasmic reticulum lumen. Involved in the correct folding of proteins and degradation of misfolded proteins via its interaction with DNAJC10/ERdj5, probably to facilitate the release of DNAJC10/ERdj5 from its substrate. Acts as a key repressor of the ERN1/IRE1-mediated unfolded protein response. In the unstressed endoplasmic reticulum, recruited by DNAJB9/ERdj4 to the luminal region of ERN1/IRE1, leading to disrupt the dimerization of ERN1/IRE1, thereby inactivating ERN1/IRE1. Accumulation of misfolded protein in the endoplasmic reticulum causes release of HSPA5/BiP from ERN1/IRE1, allowing homodimerization and subsequent activation of ERN1/IRE1. Plays an auxiliary role in post-translational transport of small presecretory proteins across endoplasmic reticulum (ER). May function as an allosteric modulator for SEC61 channel-forming translocon complex, likely cooperating with SEC62 to enable the productive insertion of these precursors into SEC61 channel. Appears to specifically regulate translocation of precursors having inhibitory residues in their mature region that weaken channel gating. May also play a role in apoptosis and cell proliferation. Plays an important role in viral binding to the host cell membrane and entry for several flaviruses such as Dengue virus, Zika virus and Japanese encephalitis virus. Acts as a component of the cellular receptor for Dengue virus serotype 2/DENV-2 on human liver cells. Acts as a receptor for CotH proteins expressed by fungi of the order mucorales, the causative agent of mucormycosis, which plays an important role in epithelial cell invasion by the fungi. Acts as a receptor for R.delemar CotH3 in nasal epithelial cells, which may be an early step in rhinoorbital/cerebral mucormycosis (RCM) disease progression.

Immunogen: Recombinant protein within human GRP78 aa 100-400.

Positive control: MCF7 cell lysate, HepG2 cell lysate, mouse brain tissue lysate, U-87 MG cell lysate, RAW264.7 cell lysate, RAW264.7 treated with 300nM Thapsigargin for 18 hours cell lysate, mouse liver tissue lysate, rat liver tissue lysate, rat pancreas tissue lysate, rat brain tissue, mouse small intestine tissue, Hela.

Subcellular location: Cytoplasm, endoplasmic reticulum lumen

Database links: SwissProt: P11021 Human | P20029 Mouse | P06761 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:200
IF-Cell	1:200

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

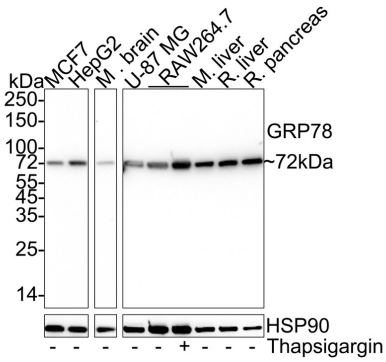

Fig1: Western blot analysis of GRP78 / BIP on different lysates with Mouse anti-GRP78 / BIP antibody (HA601076) at 1/1,000 dilution.

Lane 1: MCF7 cell lysate (15 µg/Lane)
Lane 2: HepG2 cell lysate (15 µg/Lane)
Lane 3: Mouse brain tissue lysate (30 µg/Lane)
Lane 4: U-87 MG cell lysate (30 µg/Lane)
Lane 5: RAW264.7 cell lysate (30 µg/Lane)
Lane 6: RAW264.7 treated with 300nM Thapsigargin for 18 hours cell lysate (30 µg/Lane)
Lane 7: Mouse liver tissue lysate (30 µg/Lane)
Lane 8: Rat liver tissue lysate (30 µg/Lane)
Lane 9: Rat pancreas tissue lysate (30 µg/Lane)

Predicted band size: 72 kDa
Observed band size: 72 kDa

Exposure time: Lane 1-3: 11 seconds; Lane 4-9: 4 seconds; ECL: K1801;

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 (HA601076) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

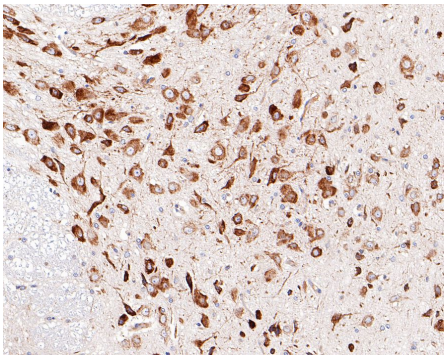

Fig2: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Mouse anti-GRP78 / BIP antibody (HA601076) at 1/200 dilution.

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 (HA601076) at 1/200 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.

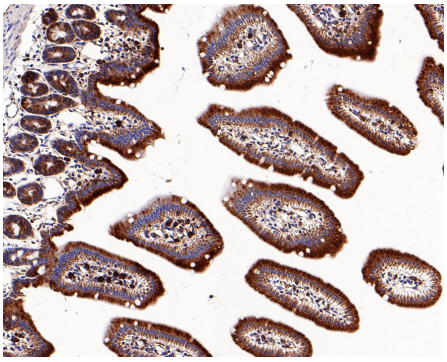

Fig3: Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue with Mouse anti-GRP78 / BIP antibody (HA601076) at 1/200 dilution.

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 (HA601076) at 1/200 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.

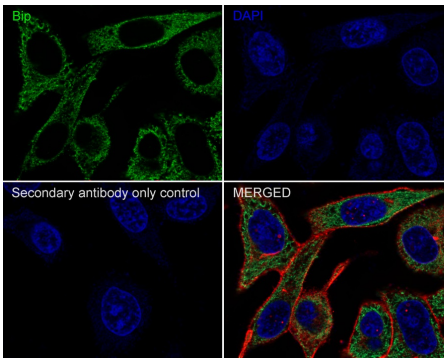

Fig4: Immunocytochemistry analysis of Hela cells labeling GRP78 / BIP with Mouse anti-GRP78 / BIP antibody (HA601076) at 1/200 dilution.

Cells were fixed in 100% methanol for 10 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 1% BSA for 30 minutes at room temperature. Cells were then incubated with Mouse anti-GRP78 / BIP antibody (HA601076) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

Background References

1. "Crystal structures of the ATPase domains of four human Hsp70 isoforms: HSPA1L/Hsp70-hom, HSPA2/Hsp70-2, HSPA6/Hsp70B', and HSPA5/BiP/GRP78." Wisniewska M., Karlberg T., Lehtio L., Johansson I., Kotenyova T., Moche M., Schuler H. PLoS ONE 5:E8625-E8625(2010)

2. "Adenosine-derived inhibitors of 78 kDa glucose regulated protein (Grp78) ATPase: insights into isoform selectivity." Macias A.T., Williamson D.S., Allen N., Borgognoni J., Clay A., Daniels Z., Dokurno P., Drysdale M.J., Francis G.L., Graham C.J., Howes R., Matassova N., Murray J.B., Parsons R., Shaw T., Surgenor A.E., Terry L., Wang Y., Wood M., Massey A.J. J. Med. Chem. 54:4034-4041(2011)