Anti-GRP78 / BIP Antibody [2-7]

M1505-13

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IF-Cell, IHC-P, IF-Tissue, FC
Molecular Wt:	Predicted band size: 78 kDa
Clone number:	2-7

Description: Binding immunoglobulin protein (BiP) also known as 78 kDa glucose-regulated protein (GRP-78) or heat shock 70 kDa protein 5 (HSPA5) is a protein that in humans is encoded by the HSPA5 gene. BiP is a HSP70 molecular chaperone located in the lumen of the endoplasmic reticulum (ER) that binds newly synthesized proteins as they are translocated into the ER, and maintains them in a state competent for subsequent folding and oligomerization. BiP is also an essential component of the translocation machinery, as well as playing a role in retrograde transport across the ER membrane of aberrant proteins destined for degradation by the proteasome. Like many stress and heat shock proteins, BiP/GRP78 has potent immunological activity when released from the internal environment of the cell into the extracelluar space.specifically, it feeds anti-inflammatory and pro-resolutory signals into immune networks, thus helping to resolve inflammation.

Immunogen: Synthetic peptide within Human GRP78 aa 605-654 / 654.

Positive control: L-929 cell 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, Hela, hybrid fish (crucian-carp) heart tissue lysates.

Subcellular location: Cytoplasm, endoplasmic reticulum lumen

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

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:250
IHC-P	1:200-1,000
IF-Tissue	1:50-1:200
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% 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: Protein A affinity purified.

Images

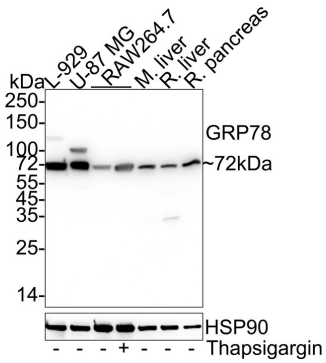


Fig1: Western blot analysis of GRP78 / BIP on different lysates with Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/1,000 dilution.

Lane 1: L-929 cell lysate
Lane 2: U-87 MG cell lysate
Lane 3: RAW264.7 cell lysate
Lane 4: RAW264.7 treated with 300nM Thapsigargin for 18 hours cell lysate
Lane 5: Mouse liver tissue lysate
Lane 6: Rat liver tissue lysate
Lane 7: Rat pancreas tissue lysate

Lysates/proteins at 30 µg/Lane.

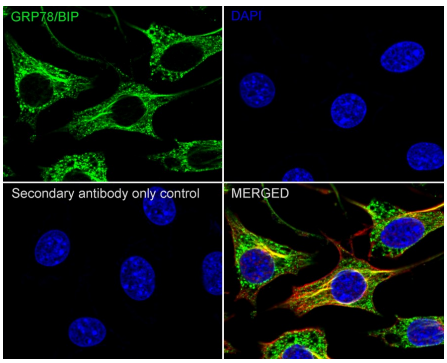
Predicted band size: 72 kDa
Observed band size: 72 kDa

Exposure time: 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 (M1505-13) 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: Immunocytochemistry analysis of L-929 cells labeling GRP78 / BIP with Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/250 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (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.

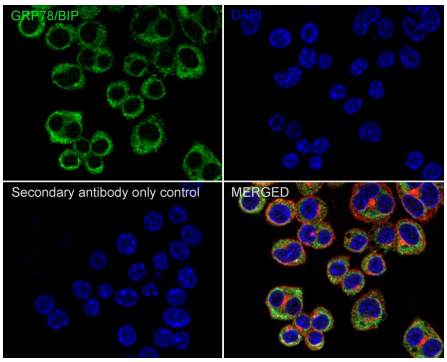


Fig3: Immunocytochemistry analysis of RAW264.7 cells labeling GRP78 / BIP with Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (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.

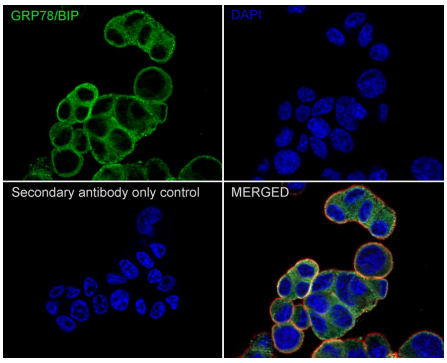


Fig4: Immunocytochemistry analysis of PC-12 cells labeling GRP78 / BIP with Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (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.

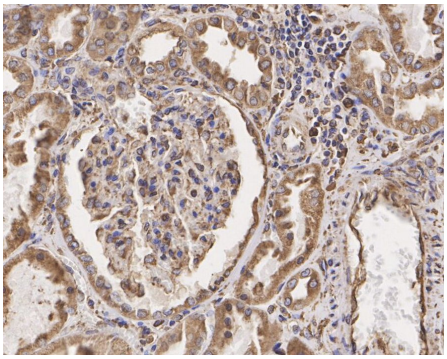


Fig5: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/1,000 dilution.

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 (M1505-13) 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.

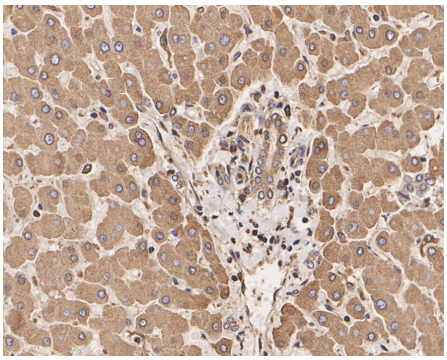


Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue with Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/1,000 dilution.

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 (M1505-13) 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.

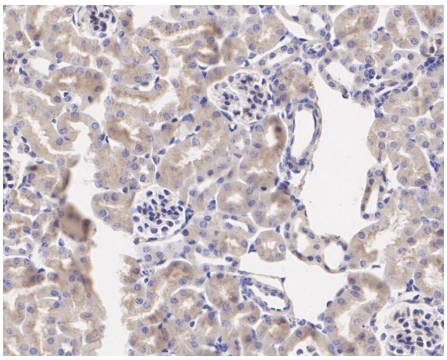


Fig7: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/1,000 dilution.

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 (M1505-13) 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.

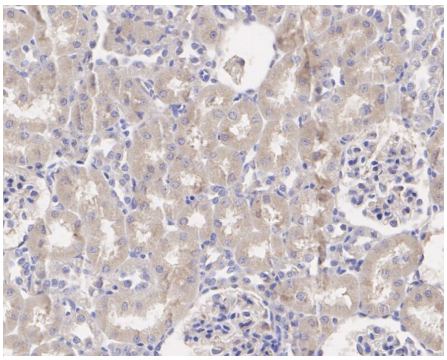


Fig8: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Mouse anti-GRP78 / BIP antibody (M1505-13) at 1/1,000 dilution.

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 (M1505-13) 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.

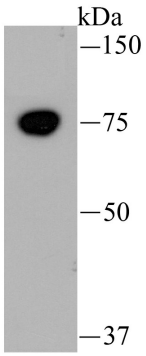


Fig9: Western blot analysis of GRP78 / BIP on hybrid fish (crucian-carp) heart tissue lysate using anti-GRP78 / BIP antibody at 1/500 dilution.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Human XTP3-B forms an endoplasmic reticulum quality control scaffold with the HRD1-SEL1L ubiquitin ligase complex and BiP." Hosokawa N., Wada I., Nagasawa K., Moriyama T., Okawa K., Nagata K. J. Biol. Chem. 283:20914-20924(2008)
2. 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)
3. 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)