Anti-Bag1 Antibody [JE63-22]

HA721885

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 38 kDa
Clone number:	JE63-22

Description: Co-chaperone for HSP70 and HSC70 chaperone proteins. Acts as a nucleotide-exchange factor (NEF) promoting the release of ADP from the HSP70 and HSC70 proteins thereby triggering client/substrate protein release. Nucleotide release is mediated via its binding to the nucleotide-binding domain (NBD) of HSPA8/HSC70 where as the substrate release is mediated via its binding to the substrate-binding domain (SBD) of HSPA8/HSC70. Inhibits the pro-apoptotic function of PPP1R15A, and has anti-apoptotic activity. Markedly increases the anti-cell death function of BCL2 induced by various stimuli.

Immunogen: Synthetic peptide within Human Bag1 aa 111-160 / 345.

Positive control: HeLa cell lysate, Jurkat cell lysate, HEK-293 cell lysate, MCF7 cell lysate, RAW264.7 cell lysate, rat testis tissue lysate, mouse testis tissue lysate, human breast cancer tissue, human prostate cancer tissue, HeLa.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: Q99933 Human | Q60739 Mouse | B0K019 Rat

Recommended Dilutions:

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

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

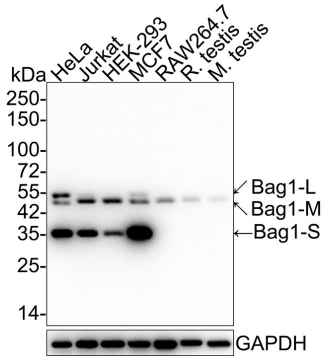

Fig1: Western blot analysis of Bag1 on different lysates with Rabbit anti-Bag1 antibody (HA721885) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: Jurkat cell lysate (20 µg/Lane)
Lane 3: HEK-293 cell lysate (20 µg/Lane)
Lane 4: MCF7 cell lysate (20 µg/Lane)
Lane 5: RAW264.7 cell lysate (20 µg/Lane)
Lane 6: Rat testis tissue lysate (40 µg/Lane)
Lane 7: Mouse testis tissue lysate (40 µg/Lane)

Predicted band size: 38 kDa
Observed band size: 35/46/52 kDa

Exposure time: 3 minutes;

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 (HA721885) 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.

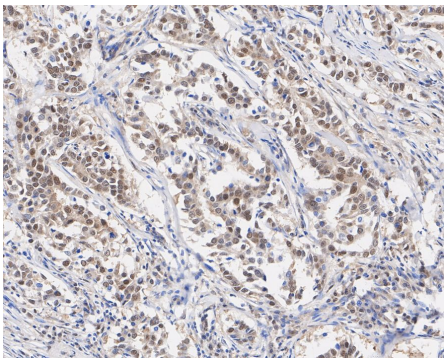

Fig2: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Bag1 antibody (HA721885) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (HA721885) at 1/500 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.

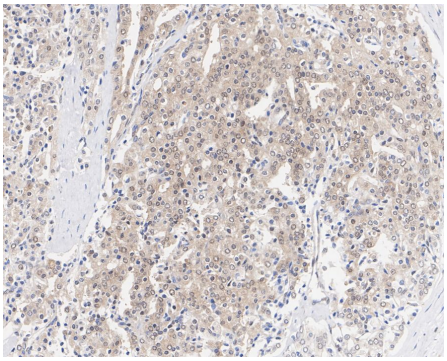

Fig3: Immunohistochemical analysis of paraffin-embedded human prostate cancer tissue with Rabbit anti-Bag1 antibody (HA721885) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (HA721885) at 1/500 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.

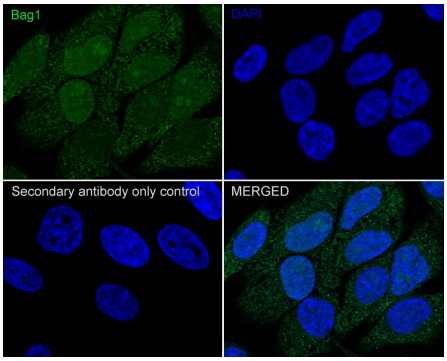

Fig4: Immunocytochemistry analysis of HeLa cells labeling Bag1 with Rabbit anti-Bag1 antibody (HA721885) at 1/100 dilution.

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 Rabbit anti-Bag1 antibody (HA721885) at 1/100 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.

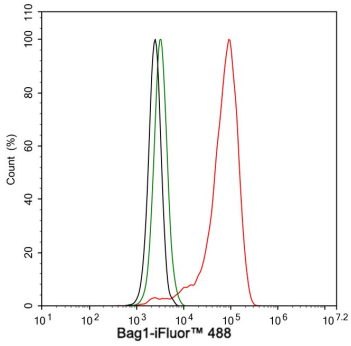

Fig5: Flow cytometric analysis of HeLa cells labeling Bag1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721885, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Rauch J.N., Zuiderweg E.R., Gestwicki J.E. Non-canonical interactions between heat shock cognate protein 70 (Hsc70) and Bcl2-associated anthanogene (BAG) co-chaperones are important for client release. J. Biol. Chem. 291:19848-19857 (2016)
2. Hung W.J., Roberson R.S., Taft J., Wu D.Y. Human BAG-1 proteins bind to the cellular stress response protein GADD34 and interfere with GADD34 functions. Mol. Cell. Biol. 23:3477-3486 (2003)