abcam

ab137076

Anti-BAT3/BAG-6 antibody [EPR9223]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR9223
Purification technique	Affinity purification Protein A
Concentration	0.381 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/10000
Notes	-

Species	Rat

Dilution info	1/1000 - 1/10000
Notes	-

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/30
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100 - 1/1000.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/50 - 1/100
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information BAG6

Function

ATP-independent molecular chaperone preventing the aggregation of misfolded and hydrophobic patches-containing proteins (PubMed:21636303). Functions as part of a cytosolic protein quality control complex, the BAG6/BAT3 complex, which maintains these client proteins in a soluble state and participates in their proper delivery to the endoplasmic reticulum or alternatively can promote their sorting to the proteasome where they undergo degradation (PubMed:20516149, PubMed:21636303, PubMed:21743475, PubMed:28104892). The BAG6/BAT3 complex is involved in the post-translational delivery of tail-anchored/type II transmembrane proteins to the endoplasmic reticulum membrane. Recruited to ribosomes, it interacts with the transmembrane region of newly synthesized tail-anchored proteins and together with SGTA and ASNA1 mediates their delivery to the endoplasmic reticulum (PubMed:20516149, PubMed:20676083, PubMed:25535373, PubMed:28104892). Client proteins that cannot be properly delivered to the endoplasmic reticulum are ubiquitinated by RNF126, an E3 ubiquitin-protein ligase associated with BAG6 and are sorted to the proteasome (PubMed:24981174, PubMed:27193484, PubMed:28104892). SGTA which prevents the recruitment of RNF126 to BAG6 may negatively regulate the ubiquitination and the proteasomal degradation of client proteins (PubMed:23129660, PubMed:25179605, PubMed:27193484). Similarly, the BAG6/BAT3 complex also functions as a sorting platform for proteins of the secretory pathway that are mislocalized to the cytosol either delivering them to the proteasome for degradation or to the endoplasmic reticulum (PubMed:21743475). The BAG6/BAT3 complex also plays a role in the endoplasmic reticulum-associated degradation (ERAD), a quality control mechanism that eliminates unwanted proteins of the endoplasmic reticulum through their retrotranslocation to the cytosol and their targeting to the proteasome. It maintains these retrotranslocated proteins in an unfolded yet soluble state condition in the cytosol to ensure their proper delivery to the proteasome (PubMed:21636303). BAG6 is also required for selective ubiquitin-mediated degradation of defective nascent chain polypeptides by the proteasome. In this context, it may participate in the production of antigenic

peptides and play a role in antigen presentation in immune response (By similarity). BAG6 is also involved in endoplasmic reticulum stress-induced pre-emptive quality control, a mechanism that selectively attenuates the translocation of newly synthesized proteins into the endoplasmic reticulum and reroutes them to the cytosol for proteasomal degradation. BAG6 may ensure the proper degradation of these proteins and thereby protects the endoplasmic reticulum from protein overload upon stress (PubMed:26565908). By inhibiting the polyubiquitination and subsequent proteasomal degradation of HSPA2 it may also play a role in the assembly of the synaptonemal complex during spermatogenesis (By similarity). Also positively regulates apoptosis by interacting with and stabilizing the proapoptotic factor AIFM1 (By similarity). By controlling the steady-state expression of the IGF1R receptor, indirectly regulates the insulin-like growth factor receptor signaling pathway (PubMed:26692333). Involved in DNA damage-induced apoptosis: following DNA damage, accumulates in the nucleus and forms a complex with p300/EP300, enhancing p300/EP300-mediated p53/TP53 acetylation leading to increase p53/TP53 transcriptional activity (PubMed:17403783). When nuclear, may also act as a component of some chromatin regulator complex that regulates histone 3 'Lys-4' dimethylation (H3K4me2) (PubMed:18765639). Released extracellularly via exosomes, it is a ligand of the natural killer/NK cells receptor NCR3 and stimulates NK cells cytotoxicity. It may thereby trigger NK cells cytotoxicity against neighboring tumor cells and immature myeloid dendritic cells (DC). Mediates ricin-induced apoptosis.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

This antibody may not be suitable for IHC with mouse or rat samples.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

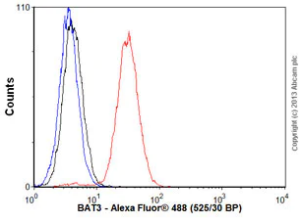
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

10 product images

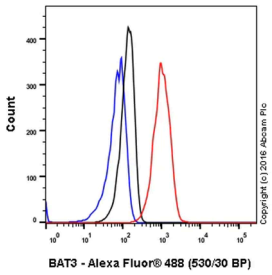


Flow Cytometry (Intracellular) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

BAT3/BAG-6 Flow Cytometry (Intracellular) staining using rabbit Anti-BAT3/BAG-6 antibody

Overlay histogram showing Hela cells stained with ab137076 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab137076 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5000 events were collected using a 20mW Argon ion laser (488nm) and

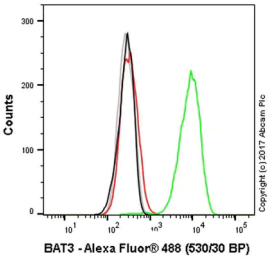
525/30 bandpass filter. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Flow Cytometry (Intracellular) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

BAT3/BAG-6 Flow Cytometry (Intracellular) staining using rabbit Anti-BAT3/BAG-6 antibody

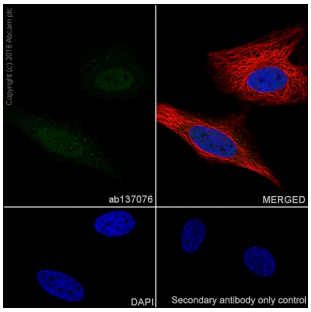
Intracellular Flow Cytometry analysis of HeLa cells labelling BAT3/BAG-6 (red) with purified ab137076 at dilution of 1/30. Goat anti rabbit IgG (Alexa Fluor®488) at 1/2000 was used as the secondary antibody. Cells were fixed with 4% paraformaldehyde. Isotype control antibody was Rabbit monoclonal IgG (black). The blue line shows cells without incubation with primary antibody and secondary antibody.



Flow Cytometry (Intracellular) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

BAT3/BAG-6 Flow Cytometry (Intracellular) staining using rabbit Anti-BAT3/BAG-6 antibody

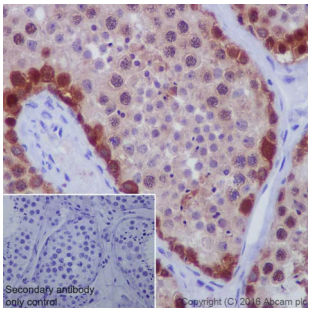
Overlay histogram showing HAP1 wildtype (green line) and HAP1-BAG6 knockout cells (red line) stained with ab137076. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1 x PBS/10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab137076 0.1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (ab150081) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line HAP1-BAG6 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This antibody can also be used in HAP1 cells fixed with 4% formaldehyde (10 min) permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

BAT3/BAG-6 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-BAT3/BAG-6 antibody

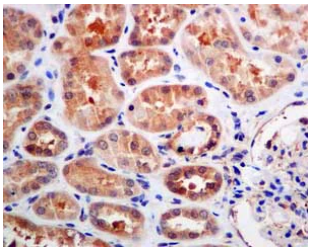
Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling BAT3/BAG-6 with purified ab137076 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077 Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counter-stained with ab195889 Anti-Alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200. DAPI (blue) was used as a nuclear counterstain. Secondary Only Control: PBS was used instead of the primary antibody as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

BAT3/BAG-6 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-BAT3/BAG-6 antibody

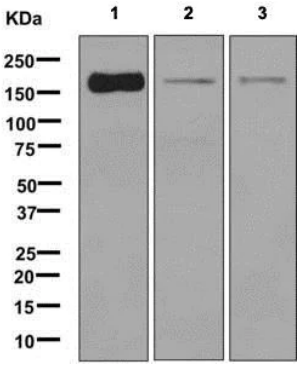
Immunohistochemical analysis of Paraffin-embedded human testis tissue sections labeling BAT3/BAG-6 with purified ab137076 at a dilution of 1/50 dilution (7.1 µg/ml). ab97051 Goat Anti-Rabbit IgG H&L (HRP) at 1/500 was used as the secondary anitbody. Sections were counterstained with hematoxylin. Antigen retrieval was heat mediated using EDTA Buffer pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

BAT3/BAG-6 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-BAT3/BAG-6 antibody

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labelling BAT3/BAG-6 with unpurified ab137076 at 1/50 dilution.



Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

BAT3/BAG-6 Western blot staining using rabbit Anti-BAT3/BAG-6 antibody

All lanes:
Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076) at 1/1000 dilution

Lane 1:
HeLa cell lysate at 10 µg

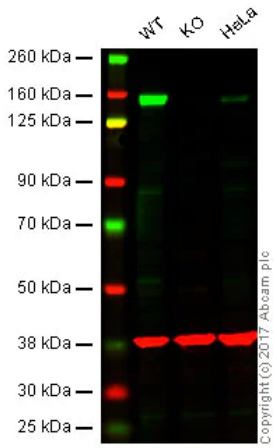
Lane 2:
A431 cell lysate at 10 µg

Lane 3:
Human fetal brain lysate at 10 µg

Secondary

All lanes:
HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 119 kDa



Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

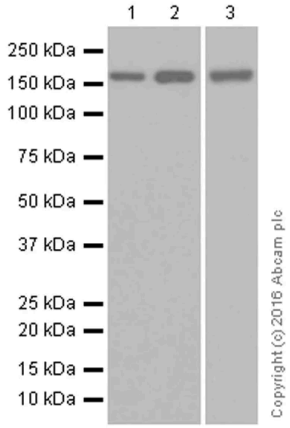
BAT3/BAG-6 Western blot staining using rabbit Anti-BAT3/BAG-6 antibody

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: BAT3/BAG-6 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lanes 1 - 3: Merged signal (red and green). Green - ab137076 observed at 155 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab137076 was shown to specifically react with BAT3/BAG-6 when BAT3/BAG-6 knockout samples were used. Wild-type and BAT3/BAG-6 knockout samples were subjected to SDS-PAGE. ab137076 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

Predicted band size: 119 kDa



Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

BAT3/BAG-6 Western blot staining using rabbit Anti-BAT3/BAG-6 antibody

Blocking/Diluting buffer 5% NFDM/TBST
All lanes:
Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076) at 1/1000 dilution

Lane 1:
A431 (Human epidermoid carcinoma cell line) whole cell lysate at 20 µg

Lane 2:
Human fetal brain tissue lysate at 20 µg

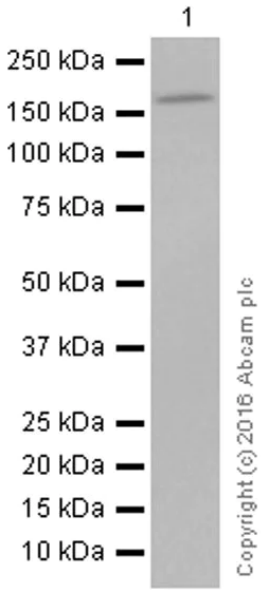
Lane 3:
Rat brain tissue lysate at 20 µg

Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 119 kDa

Observed band size: 160 kDa



Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076)

BAT3/BAG-6 Western blot staining of Mouse brain tissue lysate using rabbit Anti-BAT3/BAG-6 antibody

Blocking/Diluting buffer 5% NFDM/TBST
All lanes:
Western blot - Anti-BAT3/BAG-6 antibody [EPR9223] (ab137076) at 1/5000 dilution

All lanes:
Mouse brain tissue lysate at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 119 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.