abcam

ab32111

Anti-GRB2 antibody [Y301]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	Y301
Purification technique	Affinity purification Protein A
Specificity	ab32111 recognises GRB2. This antibody can also detect the splice isoform GRB3-3 of GRB2.
Concentration	0.313 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-

Notes	-

Expected

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

IP

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/5000
Notes	-

Species	Rat
Dilution info	1/1000 - 1/5000
Notes	-

Species	Human
Dilution info	1/1000 - 1/5000
Notes	-

ICC/IF

Tested

Species	Rat

Dilution info	1/100 - 1/250
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/40
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

Target data

See full target information GRB2

Function

Non-enzymatic adapter protein that plays a pivotal role in precisely regulated signaling cascades from cell surface receptors to cellular responses, including signaling transduction and gene expression (PubMed:11726515, PubMed:37626338). Thus, participates in many biological processes including regulation of innate and adaptive immunity, autophagy, DNA repair or necroptosis (PubMed:35831301, PubMed:37626338, PubMed:38182563). Controls signaling complexes at the T-cell antigen receptor to facilitate the activation, differentiation, and function of T-cells (PubMed:36864087, PubMed:9489702). Mechanistically, engagement of the TCR leads to phosphorylation of the adapter protein LAT, which serves as docking site for GRB2 (PubMed:9489702). In turn, GRB2 establishes a a connection with SOS1 that acts as a guanine nucleotide exchange factor and serves as a critical regulator of KRAS/RAF1 leading to MAPKs translocation to the nucleus and activation (PubMed:12171928, PubMed:25870599). Functions also a

role in B-cell activation by amplifying Ca(2+) mobilization and activation of the ERK MAP kinase pathway upon recruitment to the phosphorylated B-cell antigen receptor (BCR) (PubMed:25413232, PubMed:29523808). Plays a role in switching between autophagy and programmed necrosis upstream of EGFR by interacting with components of necrosomes including RIPK1 and with autophagy regulators SQSTM1 and BECN1 (PubMed:35831301, PubMed:38182563). Regulates miRNA biogenesis by forming a functional ternary complex with AGO2 and DICER1 (PubMed:37328606). Functions in the replication stress response by protecting DNA at stalled replication forks from MRE11-mediated degradation. Mechanistically, inhibits RAD51 ATPase activity to stabilize RAD51 on stalled replication forks (PubMed:38459011). Additionally, directly recruits and later releases MRE11 at DNA damage sites during the homology-directed repair (HDR) process (PubMed:34348893). Isoform 2. Does not bind to phosphorylated epidermal growth factor receptor (EGFR) but inhibits EGF-induced transactivation of a RAS-responsive element. Acts as a dominant negative protein over GRB2 and by suppressing proliferative signals, may trigger active programmed cell death. Mechanistically, inhibits RAS-ERK signaling and downstream cell proliferation by competing with GRB2 for SOS1 binding and thus by regulating SOS1 membrane recruitment (PubMed:36171279).

Storage

Shipped at conditions	Conditional Ambient
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

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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The GRB2 protein also known as growth factor receptor-bound protein 2 is instrumental in signal transduction. GRB2 has a molecular weight of about 25 kDa. It comprises one SH2 domain and two SH3 domains which facilitate its role in linking receptor tyrosine kinases to downstream signaling molecules. GRB2 is ubiquitously expressed in various tissues signifying its importance in diverse cellular functions.
Biological function summary	GRB2 functions as an adaptor protein that connects activated receptor tyrosine kinases to intracellular pathways. It often forms complexes with other proteins such as the SOS protein which further propagates signaling cascades critical for cell proliferation and differentiation. GRB2's ability to mediate these interactions contributes to cellular responses to external stimuli including growth factors and hormones.
Pathways	GRB2 plays an important role in the Ras-MAPK signaling pathway. It interacts with proteins like SOS and Ras enabling signal transduction from the cell surface to the nucleus which is vital for processes such as cell growth and survival. GRB2 is also implicated in the PI3K-Akt pathway connecting it to another set of signaling proteins that regulate metabolism growth and survival.
Associated diseases and disorders	GRB2 has significant associations with cancer and immune disorders. Aberrant activation of pathways involving GRB2 can lead to uncontrolled cell proliferation contributing to oncogenesis in different cancers. Additionally GRB2's interactions with proteins like BCR-ABL in chronic myeloid leukemia highlight its potential role as a therapeutic target. GRB2 inhibitors could therefore offer promising avenues for treating such conditions by disrupting its pathological signaling interactions.

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

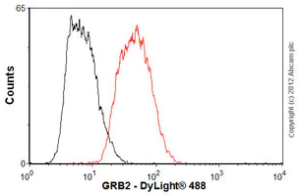
We do not recommend this combination. It is not covered by our product promise.

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.

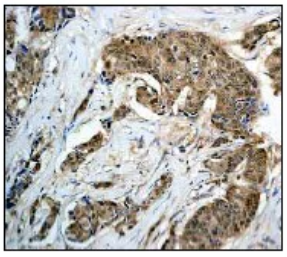
8 product images



Flow Cytometry (Intracellular) - Anti-GRB2 antibody [Y301] (ab32111)

GRB2 Flow Cytometry (Intracellular) staining using rabbit Anti-GRB2 antibody

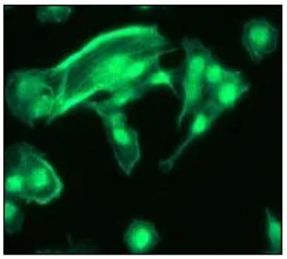
Overlay histogram showing SH-SY5Y cells stained with ab32111 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32111 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5000 events was performed.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GRB2 antibody [Y301] (ab32111)

GRB2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-GRB2 antibody

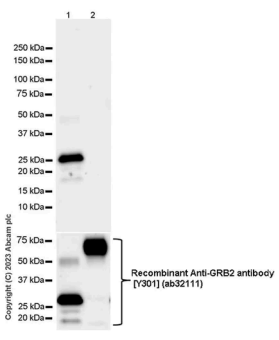
Immunohistochemical analysis of GRB2 expression in paraffin embedded human breast carcinoma tissue section using 1/100 ab32111. Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-GRB2 antibody [Y301] (ab32111)

GRB2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GRB2 antibody

Immunofluorescent analysis of GRB2 expression in PC12 cells, using 1/100 ab32111.



Western blot - Anti-GRB2 antibody [Y301] (ab32111)

Blocking and diluting buffer and concentration: 5% NFDM/TBST This antibody recognises GRB2 isoform 1 (ASH-L) and not GRB2 isoform 2 (GRB3-3).
All lanes:
Western blot - Anti-GRB2 antibody [EPR24634-17] (ab315005) at 1/1000 dilution

Lane 1:
Human GRB2 isoform 1 recombinant protein at 10 ng

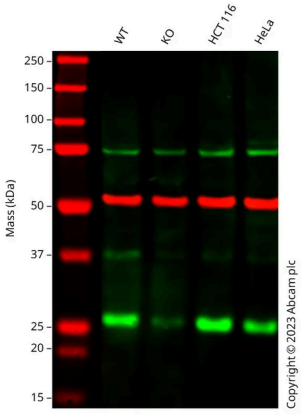
Lane 2:
Human GRB2 isoform 2 recombinant protein at 10 ng

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 25 kDa, 60 kDa

Exposure time: 92s



Western blot - Anti-GRB2 antibody [Y301] (ab32111)

GRB2 Western blot staining using rabbit Anti-GRB2 antibody

Anti-GRB2 antibody [Y301] (ab32111) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32111 was shown to bind specifically to GRB2. A band was observed at 26 kDa in wild-type A431 cell lysates with a reduction in signal observed at this size in GRB2 heterozygous knockout cell line. To generate this image, wild-type and GRB2 heterozygous knockout A431 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-GRB2 antibody [Y301] (ab32111) at 1/1000 dilution

Lane 1:
Wild-type A431 cell lysate at 20 µg

Lane 2:
GRB2 knockout A431 cell lysate at 20 µg

Lane 3:
HCT 116 cell lysate at 20 µg

Lane 4:
HeLa cell lysate at 20 µg

Secondary

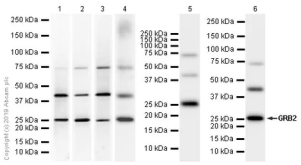
Lanes 1 - 4:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 4:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 25 kDa

Observed band size: 26 kDa



Western blot - Anti-GRB2 antibody [Y301] (ab32111)

Blocking/Diluting Buffer and concentration: 5% NFDM/TBST
We are unsure how to define the extra bands.

All lanes:
Western blot - Anti-GRB2 antibody [Y301] (ab32111) at 1/5000 dilution

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15 µg

Lane 2:
PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate at 15 µg

Lane 3:
NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 15 µg

Lane 4:
HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate at 15 µg

Lane 5:
RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 15 µg

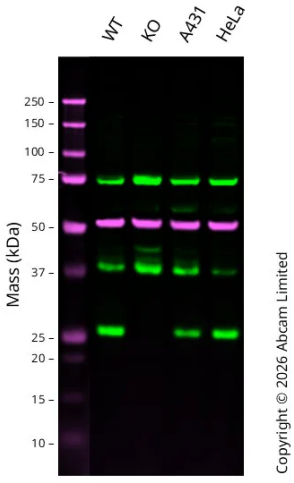
Lane 6:
C6 (Rat glial tumor glial cell) whole cell lysate at 15 µg

Secondary

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

Predicted band size: 25 kDa

Observed band size: 25 kDa



Western blot - Anti-GRB2 antibody [Y301] (ab32111)

GRB2 Western blot staining using rabbit Anti-GRB2 antibody

Western blot: Anti-GRB2 antibody [Y301] ab32111 staining at 1/2000 dilution, shown in green; Mouse anti alpha Tubulin ab7291 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 27 kDa in Wild-type HCT 116 cell lysates with no signal observed at this size in GRB2 knockout HCT 116 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween 20 (TBS-T) before incubation with primary antibodies overnight at 4 C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-GRB2 antibody [Y301] (ab32111) at 1/2000 dilution

Lane 1:
Wild-type HCT 116 at 20 µg

Lane 2:
Western blot - Human GRB2 knockout HCT116 cell line (ab273715) at 20 µg

Lane 3:
A431 at 20 µg

Lane 4:
HeLa at 20 µg

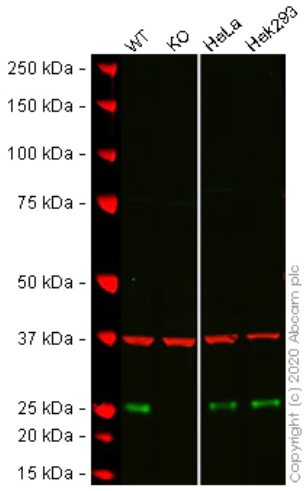
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 27 kDa

Observed band size: 27 kDa



Western blot - Anti-GRB2 antibody [Y301] (ab32111)

GRB2 Western blot staining using rabbit Anti-GRB2 antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab32111 observed at 25 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab32111 was shown to react with GRB2 in wild-type HCT 116 cells in western blot with loss of signal observed in GRB2 knockout cell line ab273715 (GRB2 knockout cell lysate ab275248). Wild-type and GRB2 knockout HCT 116 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab32111 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-GRB2 antibody [Y301] (ab32111) at 1/2000 dilution

Lane 1:
Wild-type HCT116 cell lysate at 20 µg

Lane 2:
GRB2 knockout HCT116 cell lysate at 20 µg

Lane 2:
Western blot - Human GRB2 knockout HCT116 cell line (ab273715)

Lane 3:
HeLa cell lysate at 20 µg

Lane 4:
HEK293 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 25 kDa

Observed band size: 25 kDa

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