abcam

Product datasheet

Anti-GRB2 antibody [Y301] ab32111





★★★★★ 1 Abreviews 4 References 6 Images

Overview

Product name Anti-GRB2 antibody [Y301]

Description Rabbit monoclonal [Y301] to GRB2

Host species Rabbit

Specificity ab32111 recognises GRB2. This antibody can also detect the splice isoform GRB3-3 of GRB2.

Suitable for: WB, IHC-P, ICC/IF, Flow Cyt (Intra) **Tested applications**

Unsuitable for: IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide within Human GRB2 aa 1-100 (N terminal). The exact sequence is proprietary.

Positive control Human breast carcinoma, PC12 cells WB: HCT116, HeLa, PC-12, NIH/3T3, HEK-293, RAW

264.7 and C6 cell lysates

General notes 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 production

For more information see here.

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

Properties

Form

Storage instructions Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number Y301

Isotype ΙgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab32111 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆ (1)	1/1000 - 1/5000. Predicted molecular weight: 25 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/100 - 1/250.
Flow Cyt (Intra)		1/40. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

Application notes

Is unsuitable for IP.

Target

Function Adapter protein that provides a critical link between cell surface growth factor receptors and the

Ras signaling pathway.

Isoform GRB3-3 does not bind to phosphorylated epidermal growth factor receptor (EGFR) but inhibits EGF-induced transactivation of a RAS-responsive element. Isoform GRB3-3 acts as a dominant negative protein over GRB2 and by suppressing proliferative signals, may trigger active

programmed cell death.

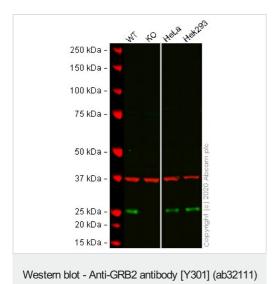
Sequence similarities Belongs to the GRB2/sem-5/DRK family.

> Contains 1 SH2 domain. Contains 2 SH3 domains.

Domain The SH3 domains mediate interaction with RALGPS1 and SHB.

Cellular localization Golgi apparatus.

Images



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

Lane 1: Wild-type HCT116 cell lysate

Lane 2: GRB2 knockout HCT116 cell lysate

Lane 3 : HeLa cell lysate

Lane 4 : HEK293 cell lysate

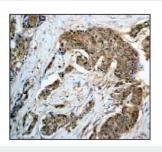
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 25 kDa

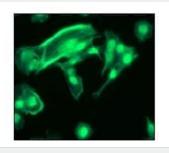
Lanes 1 - 4: Merged signal (red and green). Green - ab32111 observed at 25 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab32111 was shown to react with GRB2 in HCT 116 wild-type cells in western blot with loss of signal observed in GRB2 knockout cell line ab273715 (GRB2 knockout cell lysate ab275248). HCT 116 wild-type and GRB2 knockout 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 lgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

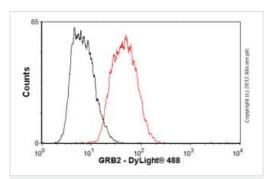


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GRB2 antibody [Y301] (ab32111)

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.

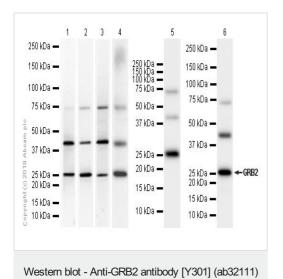


Immunocytochemistry/ Immunofluorescence - Anti-GRB2 antibody [Y301] (ab32111) Immunofluorescent analysis of GRB2 expression in PC12 cells, using 1/100 ab32111.



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

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 lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1 μ g/1x106 cells) used under the same conditions. Acquisition of >5,000 events was performed.



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

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates

Lane 3: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

Lane 4: HEK-293 (Human embryonic kidney epithelial cell) whole
cell lysates

Lane 5: RAW 264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysates

Lane 6: C6 (Rat glial tumor glial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

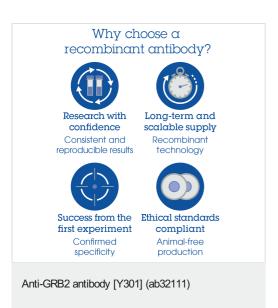
Secondary

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

Predicted band size: 25 kDa
Observed band size: 25 kDa

Blocking/Diluting Buffer and concentration: 5% NFDM/TBST

We are unsure how to define the extra bands.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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