abcam

Product datasheet

# Anti-GRB2 antibody [Y301] ab32111

KO 評価済 リコンビナント RabMAb

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## 製品の概要

| | |
|---|---|
| **製品名** | Anti-GRB2 antibody [Y301] |
| **製品の詳細** | Rabbit monoclonal [Y301] to GRB2 |
| **由来種** | Rabbit |
| **特異性** | ab32111 recognises GRB2. This antibody can also detect the splice isoform GRB3-3 of GRB2. |
| **アプリケーション** | **適用あり:** WB, IHC-P, ICC/IF, Flow Cyt (Intra)<br>**適用なし:** IP |
| **種交差性** | **交差種:** Mouse, Rat, Human |
| **免疫原** | Synthetic peptide within Human GRB2 aa 1-100 (N terminal). The exact sequence is proprietary. |
| **ポジティブ・コントロール** | Human breast carcinoma, PC12 cells WB: HCT116, HeLa, PC-12, NIH/3T3, HEK-293, RAW 264.7 and C6 cell lysates |
| **特記事項** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. |

## 製品の特性

| | |
|---|---|
| **製品の状態** | Liquid |
| **保存方法** | Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles. |
| **バッファー** | pH: 7.20<br>Preservative: 0.01% Sodium azide<br>Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA |
| **精製度** | Protein A purified |
| **ポリ/モノ** | モノクローナル |
| **クローン名** | Y301 |

**アイソタイプ** IgG

## アプリケーション

**The Abpromise guarantee** **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab32111の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション | Abreviews | 特記事項 |
|---|---|---|
| **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.<br>**ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |

**追加情報** Is unsuitable for IP.

## ターゲット情報

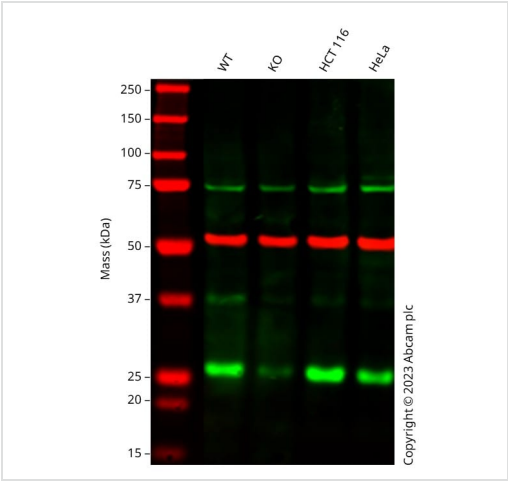
**機能** Adapter protein that provides a critical link between cell surface growth factor receptors and the Ras signaling pathway.
Isoform GRB3-3 does not bind to phosphorylated epidermal growth factor receptor (EGFR) but inhibits EGF-induced transactivation of a RAS-responsive element. Isoform GRB3-3 acts as a dominant negative protein over GRB2 and by suppressing proliferative signals, may trigger active programmed cell death.

**配列類似性** Belongs to the GRB2/sem-5/DRK family.
Contains 1 SH2 domain.
Contains 2 SH3 domains.

**ドメイン** The SH3 domains mediate interaction with RALGPS1 and SHB.

**細胞内局在** Golgi apparatus.

## 画像




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

**All lanes :** Anti-GRB2 antibody [Y301] (ab32111) at 1/1000 dilution

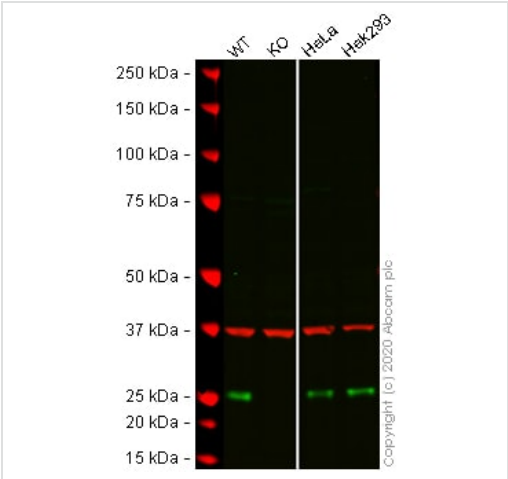
**Lane 1 :** Wild-type A431 cell lysate
**Lane 2 :** GRB2 knockout A431 cell lysate
**Lane 3 :** HCT 116 cell lysate
**Lane 4 :** HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 25 kDa
**Observed band size:** 26 kDa

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.




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

**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

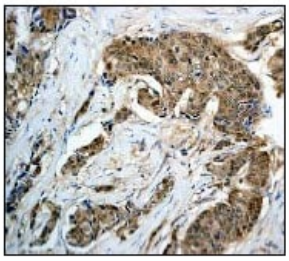
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

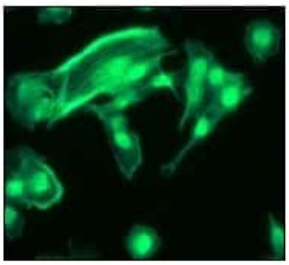
**Lanes 1 - 4:** Merged signal (red and green). Green - ab32111 observed at 25 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

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 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.



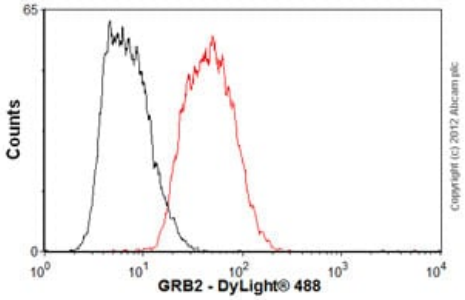
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GRB2 antibody [Y301] (ab32111)

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.



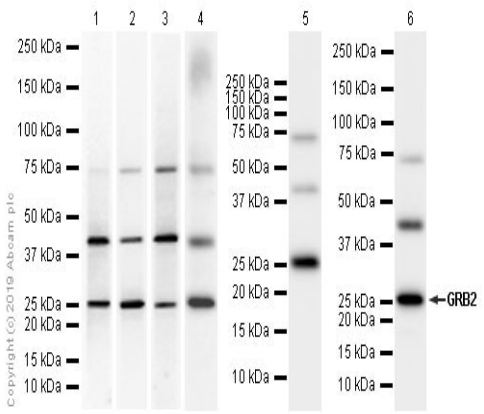
Immunocytochemistry/ Immunofluorescence - Anti-GRB2 antibody [Y301] (ab32111)

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 IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.




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

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

**Lane 1 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
**Lane 2 :** PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate
**Lane 3 :** NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
**Lane 4 :** HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate
**Lane 5 :** RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
**Lane 6 :** C6 (Rat glial tumor glial cell) whole cell lysate

Lysates/proteins at 15 µg per lane.

**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) 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.


Anti-GRB2 antibody [Y301] (ab32111)

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

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