abcam

# ab227117

# Anti-GRB2 antibody [Y237] - BSA and Azide free

Recombinant | RabMAb | KO Validated

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Constituents: PBS |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Carrier free** | Yes |
| **Clone number** | Y237 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 1.018 mg/mL The concentration of this product may be batch-dependent<br>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** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human, Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

### Expected

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

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody. |

### Expected

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

## IP

### Expected

| | |
|---|---|
| **Species** | Human, Rat, Mouse |
| **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** | Blue Ice |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | +4°C |
| **Storage information** | Do Not Freeze |

## Notes

ab227117 is the carrier-free version of ab32037.

**Conjugation ready**
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

**Compatibility**
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

## 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**

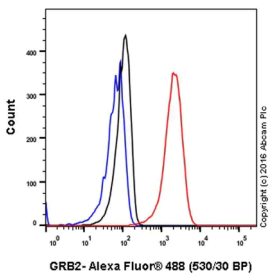
We do not recommend this combination. It is not covered by our product promise.

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.

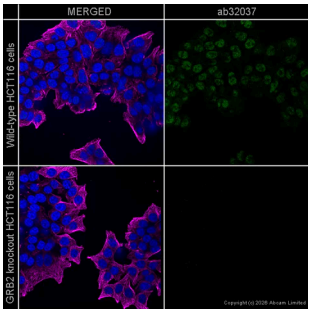
## 7 product images




## Flow Cytometry (Intracellular) - Anti-GRB2 antibody [Y237] - BSA and Azide free (ab227117)

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

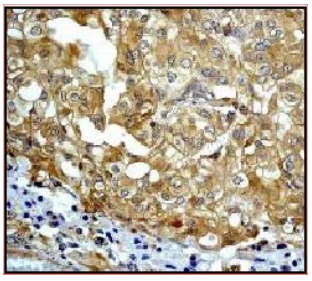
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling GRB2 (red) with ab32037 at a 1/20 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (Black) (ab172730). Blue (unlabeled control) - Cell without incubation with primary antibody and secondary antibody (Blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32037).




## Immunocytochemistry/ Immunofluorescence - Anti-GRB2 antibody [Y237] - BSA and Azide free (ab227117)

GRB2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GRB2 antibody

This data was developed using ab32037 the same antibody clone in a different buffer formulation. ab32037 staining GRB2 in Wild-type HCT116 (positive) and HCT116-GRB2 knockout (KO) (negative) cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated for 2 hours at room temperature with ab32037 at 0.2µg/ml (shown in green) and ab7291 Anti-alpha Tubulin antibody [DM1A] - Loading Control (shown in pseudocolour magenta). Cells were then incubated with ab150081, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution and ab150120, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution for 1 hour at room temperature. Nuclear DNA was labeled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Revvity) and a maximum intensity projection of confocal sections is shown.

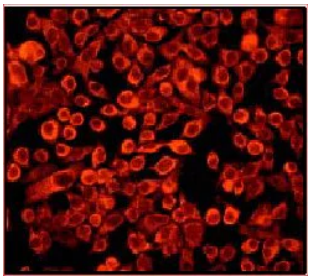


## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GRB2 antibody [Y237] - BSA and Azide free (ab227117)

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

This IHC data was generated using the same anti-GRB2 antibody clone, Y237, in a different buffer formulation (cat# ab32037).
Immunohistochemical analysis of GRB2 expression in paraffin embedded human ovarian carcinoma, using 1/100 ab32037.

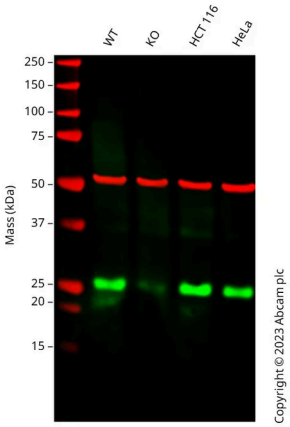
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



## Immunocytochemistry/ Immunofluorescence - Anti-GRB2 antibody [Y237] - BSA and Azide free (ab227117)

GRB2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-GRB2 antibody

Immunofluorescent analysis of GRB2 expression in HeLa cells using 1/50 ab32037.This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32037).




## Western blot - Anti-GRB2 antibody [Y237] - BSA and Azide free (ab227117)

GRB2 Western blot staining using rabbit Anti-GRB2 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab32037). Anti-GRB2 antibody [Y237] (ab32037) staining at 1/5000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32037 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 [Y237] (ab32037) at 1/5000 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

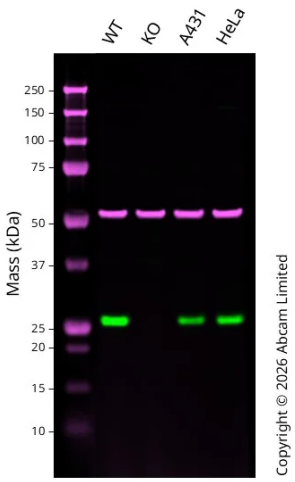
Lanes 1 - 4:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

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 [Y237] - BSA and Azide free (ab227117)

GRB2 Western blot staining using rabbit Anti-GRB2 antibody

This data was developed using ab32037, the same antibody clone in a different buffer formulation.

Western blot: Anti-GRB2 antibody [Y237] ab32037 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 [Y237] (ab32037) 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

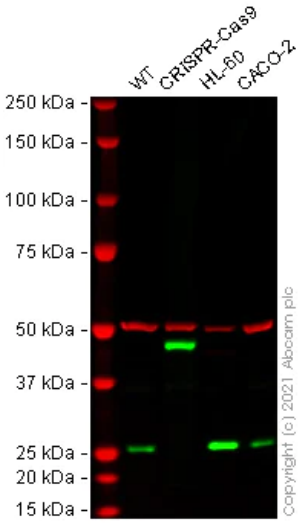
Secondary

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 [Y237] - BSA and Azide free (ab227117)

This data was developed using the same antibody clone in a different buffer formulation (ab32037).
**Lanes 1 - 4:** Merged signal (red and green). Green - ab32037 observed at 25 kDa. Red - loading control, ab7291 (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab32037 was shown to react with GRB2 in western blot. The band observed in the CRISPR-Cas9 edited cell lysate lane above 25 kDa is likely to represent an insertion. This has not been investigated further and the functional properties of the gene product have not been determined. Cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab32037 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 5000 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 h at room temperature before imaging.

All lanes:
Western blot - Anti-GRB2 antibody [Y237] (ab32037) at 1/5000 dilution

Lane 1:
Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 2:
GRB2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 3:
HL-60 (Human promyelocytic leukemia cell line) whole cell lysate at 20 µg

Lane 4:
Caco-2 (Human colorectal adenocarcinoma cell line) whole 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'.