Anti-GRB2 Antibody [JE49-73]

HA721923

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 25 kDa
Clone number:	JE49-73

Description: The protein encoded by this gene binds the epidermal growth factor receptor and contains one SH2 domain and two SH3 domains. Its two SH3 domains direct complex formation with proline-rich regions of other proteins, and its SH2 domain binds tyrosine phosphorylated sequences. This gene is similar to the Sem5 gene of C.elegans, which is involved in the signal transduction pathway. Two alternatively spliced transcript variants encoding different isoforms have been found for this gene. Adapter protein that provides a critical link between cell surface growth factor receptors and the Ras signaling pathway.

Immunogen: Recombinant protein within Human GRB2 aa 1-100 / 217.

Positive control: HeLa cell lysate, 293T cell lysate, A431 cell lysate, MCF7 cell lysate, HCT 116 cell lysate, COS-1 cell lysate, RAW264.7 cell lysate, NIH/3T3 cell lysate, C6 cell lysate, PC-12 cell lysate, human lung tissue lysate, mouse testis tissue lysate, rat testis tissue lysate, PC-12, MCF7, RAW264.7.

Subcellular location: Cytoplasm. Endosome. Golgi apparatus. Nucleus.

Database links: SwissProt: P62993 Human | Q60631 Mouse | P62994 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

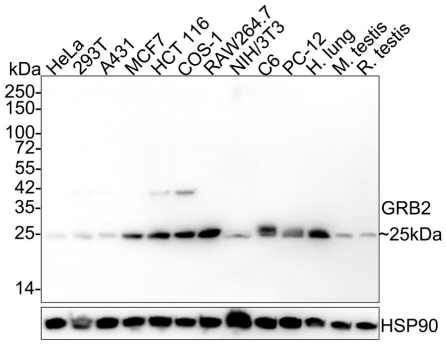

Fig1: Western blot analysis of GRB2 on different lysates with Rabbit anti-GRB2 antibody (HA721923) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: 293T cell lysate
Lane 3: A431 cell lysate
Lane 4: MCF7 cell lysate
Lane 5: HCT 116 cell lysate
Lane 6: COS-1 cell lysate
Lane 7: RAW264.7 cell lysate
Lane 8: NIH/3T3 cell lysate
Lane 9: C6 cell lysate
Lane 10: PC-12 cell lysate
Lane 11: Human lung tissue lysate
Lane 12: Mouse testis tissue lysate
Lane 13: Rat testis tissue lysate

Lysates/proteins at 30 µg/Lane.

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

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721923) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

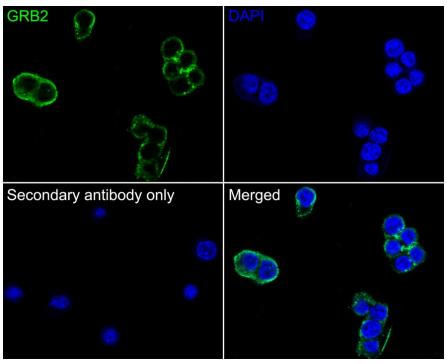

Fig2: Immunocytochemistry analysis of PC-12 cells labeling GRB2 with Rabbit anti-GRB2 antibody (HA721923) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GRB2 antibody (HA721923) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

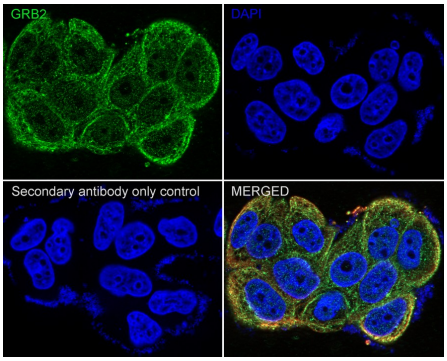

Fig3: Immunocytochemistry analysis of MCF7 cells labeling GRB2 with Rabbit anti-GRB2 antibody (HA721923) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GRB2 antibody (HA721923) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

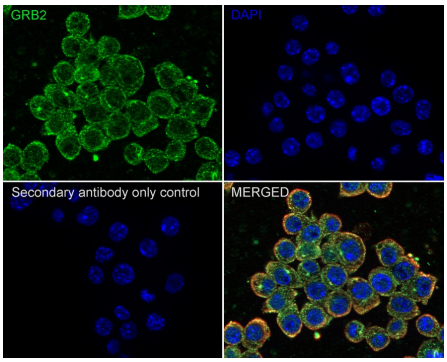

Fig4: Immunocytochemistry analysis of RAW264.7 cells labeling GRB2 with Rabbit anti-GRB2 antibody (HA721923) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GRB2 antibody (HA721923) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

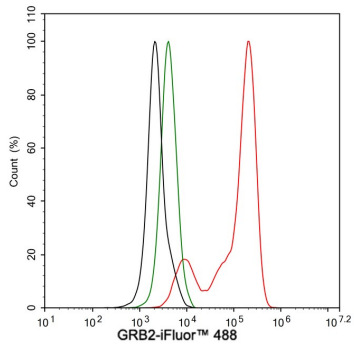

Fig5: Flow cytometric analysis of MCF7 cells labeling GRB2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721923, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

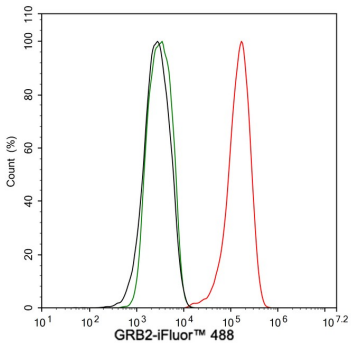

Fig6: Flow cytometric analysis of PC-12 cells labeling GRB2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721923, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Lowenstein E.J., Daly R.J., Batzer A.G., Li W., Margolis B., Lammers R., Ullrich A., Skolnik E.Y., Bar-Sagi D., Schlessinger J. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. Cell 70:431-442 (1992).
2. Pao-Chun L., Chan P.M., Chan W., Manser E. Cytoplasmic ACK1 interaction with multiple receptor tyrosine kinases is mediated by Grb2: an analysis of ACK1 effects on Axl signaling. J. Biol. Chem. 284:34954-34963 (2009).