Anti-GRASP65 Antibody [JB36-34]

ET7107-13

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 46 kDa
Clone number:	JB36-34

Description: Golgi reassembly-stacking protein of 65 kDa (GRASP65) also known as Golgi reassembly-stacking protein 1 (GORASP1) is a protein that in humans is encoded by the GORASP1 gene. The Golgi complex plays a key role in the sorting and modification of proteins exported from the endoplasmic reticulum. The GRASP65 protein is a peripheral membrane protein anchored to the lipid bilayer through myristoylation of a glycine residue near the protein's amino terminus. It is involved in establishing the stacked structure of the Golgi apparatus and linking the stacks into larger ribbons in vertebrate cells. It is a caspase-3 substrate, and cleavage of this encoded protein contributes to Golgi fragmentation in apoptosis. GRASP65 can form a complex with the Golgi matrix protein GM130, and this complex binds to the vesicle docking protein p115. Several alternatively spliced transcript variants of this gene have been identified, but their full-length natures have not been determined.

Immunogen: Synthetic peptide within Human GRASP65 aa 396-440 / 440.

Positive control: SK-Br-3 cell lysates, HepG2, HUVEC, MCF-7, human colon carcinoma tissue, human uterus tissue.

Subcellular location: Golgi apparatus.

Database links: SwissProt: Q9BQQ3 Human

Recommended Dilutions:

WB	1:500-1:1,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
FC	1:50-1:100
IP	Use at an assay dependent concentration.

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

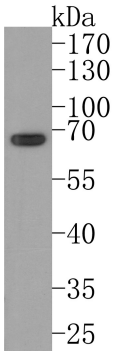

Fig1: Western blot analysis of GRASP65 on SK-Br-3 cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET7107-13, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

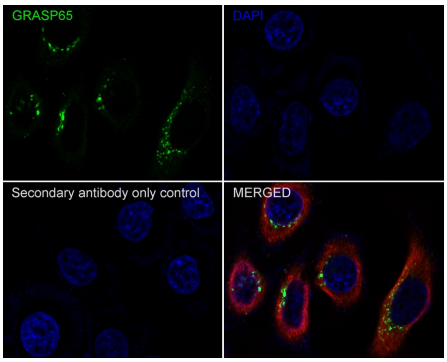

Fig2: Immunocytochemistry analysis of HepG2 cells labeling GRASP65 with Rabbit anti-GRASP65 antibody (ET7107-13) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-GRASP65 antibody (ET7107-13) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. 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.

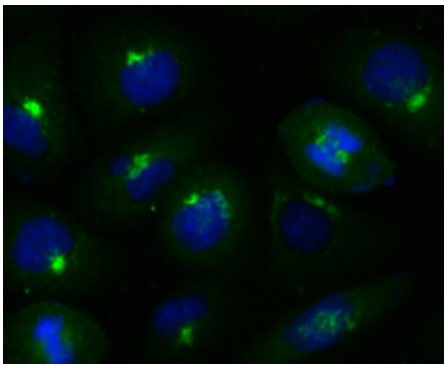

Fig3: ICC staining of GRASP65 in HUVEC cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7107-13, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

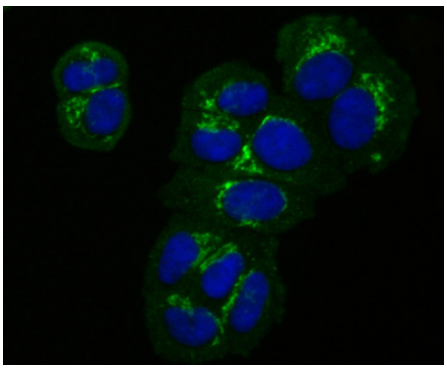

Fig4: ICC staining of GRASP65 in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7107-13, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

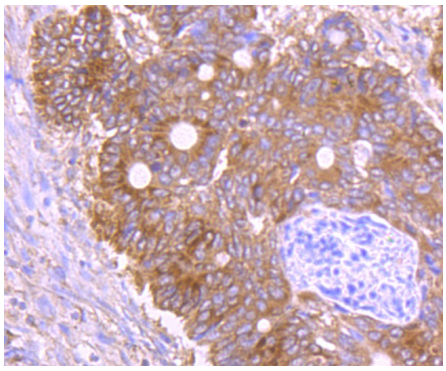

Fig5: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-GRASP65 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-13, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

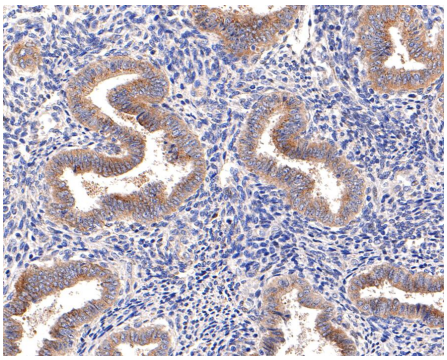

Fig6: Immunohistochemical analysis of paraffin-embedded human uterus tissue with Rabbit anti-GRASP65 antibody (ET7107-13) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-13) at 1/100 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

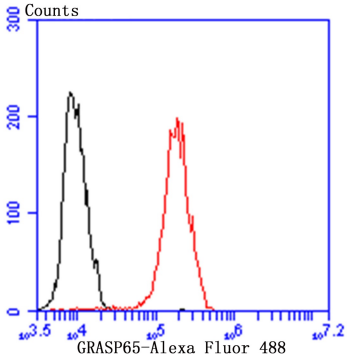

Fig7: Flow cytometric analysis of GRASP65 was done on MCF-7 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET7107-13, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.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. Puthenveedu M.A. et al. GM130 and GRASP65-dependent lateral cisternal fusion allows uniform Golgi-enzyme distribution. Nat. Cell Biol. 8:238-248(2006).
2. Marra P. et al. The GM130 and GRASP65 Golgi proteins cycle through and define a subdomain of the intermediate compartment. Nat. Cell Biol. 3:1101-1113(2001).