

GRASP65 (B-3): sc-398363

BACKGROUND

The Golgi apparatus is a highly complex organelle comprised of a stack of cisternal membranes on the secretory pathway from the ER to the cell surface. The structure is maintained by an exoskeleton or Golgi matrix constructed from a family of coiled-coil protein, the golgins and other peripheral membrane components such as GRASP55 and GRASP65. GRASP55 (Golgi reassembly stacking protein or p59) is a component of the Golgi stacking machinery. GRASP55 is highly homologous to GRASP65 and contains two PDZ domains. GRASP55 is myristoylated and palmitoylated. Unlike GRASP65, GRASP55 does not have detectable binding with the vesicle docking protein GM130 and is located on the medial-Golgi rather than *cis*-Golgi. Both GRASP55 and GRASP65 function in the stacking of Golgi cisternae. The novel coiled-coil protein golgin 45 interacts with GRASP55 and the GTP form of Rab 2, suggesting that GRASP55 and golgin 45 form a Rab 2 effector complex on medial-Golgi essential for normal protein transport and Golgi structure. ERK2 directly phosphorylates GRASP55, which is phosphorylated in mitotic cells, suggesting that mitogen-activated protein kinase kinase (MKK)/ERK pathway phosphorylates the Golgi during mitosis.

REFERENCES

1. Barr, F.A., et al. 1997. GRASP65, a protein involved in the stacking of Golgi cisternae. *Cell* 91: 253-262.
2. Shorter, J., et al. 1999. GRASP55, a second mammalian GRASP protein involved in the stacking of Golgi cisternae in a cell-free system. *EMBO J.* 18: 4949-4960.
3. Short, B., et al. 2001. A GRASP55-Rab 2 effector complex linking Golgi structure to membrane traffic. *J. Cell Biol.* 155: 877-883.
4. Barr, F.A., et al. 2001. Golgi matrix proteins interact with p24 cargo receptors and aid their efficient retention in the Golgi apparatus. *J. Cell Biol.* 155: 885-891.
5. Sutterlin, C., et al. 2001. Polo-like kinase is required for the fragmentation of pericentriolar Golgi stacks during mitosis. *Proc. Natl. Acad. Sci. USA* 98: 9128-9132.

CHROMOSOMAL LOCATION

Genetic locus: GORASP1 (human) mapping to 3p22.2; Gorasp1 (mouse) mapping to 9 F4.

SOURCE

GRASP65 (B-3) is a mouse monoclonal antibody specific for an epitope mapping between amino acids 2-29 at the N-terminus of GRASP65 of human origin.

PRODUCT

Each vial contains 200 µg IgG_{2a} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-398363 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

APPLICATIONS

GRASP65 (B-3) is recommended for detection of GRASP65 of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for GRASP65 siRNA (h): sc-41228, GRASP65 siRNA (m): sc-41229, GRASP65 shRNA Plasmid (h): sc-41228-SH, GRASP65 shRNA Plasmid (m): sc-41229-SH, GRASP65 shRNA (h) Lentiviral Particles: sc-41228-V and GRASP65 shRNA (m) Lentiviral Particles: sc-41229-V.

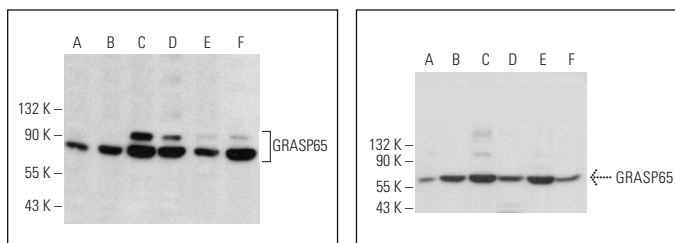
Molecular Weight of GRASP65: 65 kDa.

Positive Controls: A549 cell lysate: sc-2413, MCF7 whole cell lysate: sc-2206 or C6 whole cell lysate: sc-364373.

RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

DATA



GRASP65 (B-3): sc-398363. Western blot analysis of GRASP65 expression in MCF7 (A), HT-29 (B), 3T3-L1 (C), EOC 20 (D), PC-12 (E) and C6 (F) whole cell lysates.

GRASP65 (B-3): sc-398363. Western blot analysis of GRASP65 expression in Hep G2 (A), A549 (B), SK-BR-3 (C), OVCAR-3 (D), NIH/3T3 (E) and K-562 (F) whole cell lysates.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.