

GRASP65 (h): 293T Lysate: sc-117395

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., Puype, M., Vandekerckhove, J. and Warren, G. 1997. GRASP65, a protein involved in the stacking of Golgi cisternae. *Cell* 91: 253-262.
2. Shorter, J., Watson, R., Giannakou, M.E., Clarke, M., Warren, G. and Barr, F.A. 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. Barr, F.A., Preisinger, C., Kopajtich, R. and Korner, R. 2001. Golgi matrix proteins interact with p24 cargo receptors and aid their efficient retention in the Golgi apparatus. *J. Cell Biol.* 155: 885-891.
4. Sutterlin, C., Lin, C.Y., Feng, Y., Ferris, D.K., Erikson, R.L. and Malhotra, V. 2001. Polo-like kinase is required for the fragmentation of pericentriolar Golgi stacks during mitosis. *Proc. Natl. Acad. Sci. USA* 98: 9128-9132.
5. Moyer, B.D., Alan, B.B. and Balch, W.E. 2001. Rab 1 interaction with a GM130 effector complex regulates COPII vesicle *cis*-Golgi tethering. *Traffic* 2: 268-276.
6. Yoshimura, S.I., Nakamura, N., Barr, F.A., Misumi, Y., Ikehara, Y., Ohno, H., Sakaguchi, M. and Mihara, K. 2001. Direct targeting of *cis*-Golgi matrix proteins to the Golgi apparatus. *J. Cell Sci.* 114: 4105-4115.

CHROMOSOMAL LOCATION

Genetic locus: GORASP1 (human) mapping to 3p22.2.

PRODUCT

GRASP65 (h): 293T Lysate represents a lysate of human GRASP65 transfected 293T cells and is provided as 100 µg protein in 200 µl SDS-PAGE buffer.

STORAGE

Store at -20° C. Repeated freezing and thawing should be minimized. Sample vial should be boiled once prior to use. Non-hazardous. No MSDS required.

APPLICATIONS

GRASP65 (h): 293T Lysate is suitable as a Western Blotting positive control for human reactive GRASP65 antibodies. Recommended use: 10-20 µl per lane.

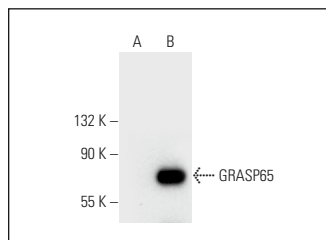
Control 293T Lysate: sc-117752 is available as a Western Blotting negative control lysate derived from non-transfected 293T cells.

GRASP65 (B-3): sc-398363 is recommended as a positive control antibody for Western Blot analysis of enhanced human GRASP65 expression in GRASP65 transfected 293T cells (starting dilution 1:100, dilution range 1:100-1:1,000).

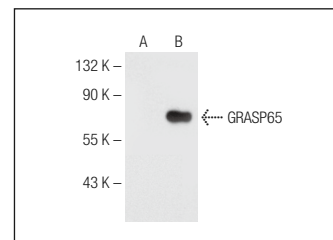
RECOMMENDED SUPPORT REAGENTS

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.

DATA



GRASP65 (B-3): sc-398363. Western blot analysis of GRASP65 expression in non-transfected: sc-117752 (A) and human GRASP65 transfected: sc-117395 (B) 293T whole cell lysates.



GRASP65 (D-12): sc-374423. Western blot analysis of GRASP65 expression in non-transfected: sc-117752 (A) and human GRASP65 transfected: sc-117395 (B) 293T whole cell lysates.

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.