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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



Snapin shRNA (m) Lentiviral Particles: sc-45546-V

BACKGROUND

Biogenesis of lysosome-related organelles complex-1 (BLOC-1) is a multi-subunit protein necessary for biogenesis of specialized organelles of the endosomal-lysosomal system (such as melanosomes and platelet-dense granules). The complex consists of coiled-coil-forming proteins Snapin, Pallidin, Cappuccino, Muted, BLOS1, BLOS2 and BLOS3. The localization of these proteins varies as they can be cytoplasmic, peripheral membrane bound or anchored to the vesicular membrane. Snapin associates with the SNARE complex in neurons. Phosphorylation of Snapin increases its interaction with SNAP25 and affects exocytotic protein interactions. Snapin interacts with the N-terminus of regulator of G protein signaling 7 (RGS7), which is important in synaptic vesicle exocytosis.

REFERENCES

1. Ilardi, J.M., et al. 1999. Snapin: a SNARE-associated protein implicated in synaptic transmission. *Nat. Neurosci.* 2: 119-124.
2. Hunt, R.A., et al. 2003. Snapin interacts with the N-terminus of regulator of G protein signaling 7. *Biochem. Biophys. Res. Commun.* 303: 594-549.
3. Buxton, P., et al. 2003. Identification and characterization of Snapin as a ubiquitously expressed SNARE-binding protein that interacts with SNAP23 in non-neuronal cells. *Biochem. J.* 375: 433-440.
4. Starcevic, M., et al. 2004. Identification of Snapin and three novel proteins (BLOS1, BLOS2, and BLOS3/reduced pigmentation) as subunits of biogenesis of lysosome-related organelles complex-1 (BLOC-1). *J. Biol. Chem.* 279: 28393-28401.
5. Chou, J.L., et al. 2005. Regulation of type VI adenylyl cyclase by Snapin, a SNAP25-binding protein. *J. Biol. Chem.* 27: 46271-46279.

CHROMOSOMAL LOCATION

Genetic locus: Snapin (mouse) mapping to 3 F1.

PRODUCT

Snapin shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Snapin siRNA (m): sc-45546 and Snapin shRNA Plasmid (m): sc-45546-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

Snapin shRNA (m) Lentiviral Particles is recommended for the inhibition of Snapin expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Snapin (FL-136): sc-366870 is recommended as a control antibody for monitoring of Snapin gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Snapin gene expression knockdown using RT-PCR Primer: Snapin (m)-PR: sc-45546-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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