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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) 

PSD-95 siRNA (h): sc-42010

BACKGROUND

The *Drosophila* discs large (dlg) tumor suppressor gene was first identified in *Drosophila* through genetic analysis of germline mutations. Several mammalian homologs were subsequently identified and categorized into a protein family termed MAGUK (membrane-associated guanylate kinase homolog). Human homologs of dlg include hdlg-1 (rat SAP 97) and NE-dlg (neuronal and endocrine dlg). The rat synaptic protein PSD-95 (also designated SAP 90) also shares homology with these proteins. MAGUKs are localized at the membrane-cytoskeleton interface and contain several distinct domains which suggest a role for these proteins in intracellular signal transduction. Interaction of hdlg-1 and NE-dlg with the tumor suppressor protein APC suggest that MAGUK proteins may also play a role in regulation of growth.

REFERENCES

1. Gateff, E. and Mechler, B.M. 1989. Tumor-suppressor genes of *Drosophila melanogaster*. *Crit. Rev. Oncog.* 1: 221-245.
2. Cho, K.O., et al. 1992. The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein. *Neuron* 9: 929-942.
3. Stehle, T., et al. 1992. Refined structure of the complex between guanylate kinase and its substrate GMP at 2.0 Å resolution. *J. Mol. Biol.* 224: 1127-1141.
4. Woods, D.F. and Bryant, P.J. 1993. ZO-1, DlgA and PSD-95/SAP 90: homologous proteins in tight, septate and synaptic cell junctions. *Mech. Dev.* 44: 85-89.
5. Lue, R.A., et al. 1994. Cloning and characterization of hdlg: the human homologue of the *Drosophila* discs large tumor suppressor binds to protein 4.1. *Proc. Natl. Acad. Sci. USA* 91: 9818-9822.
6. Müller, B.M., et al. 1995. Molecular characterization and spatial distribution of SAP 97, a novel presynaptic protein homologous to SAP 90 and the *Drosophila* discs large tumor suppressor protein. *J. Neurosci.* 15: 2354-2356.
7. Matsumine, A., et al. 1996. Binding of APC to the human homolog of the *Drosophila* discs large tumor suppressor protein. *Science* 272: 1020-1023.

CHROMOSOMAL LOCATION

Genetic locus: DLG4 (human) mapping to 17p13.1.

PRODUCT

PSD-95 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PSD-95 shRNA Plasmid (h): sc-42010-SH and PSD-95 shRNA (h) Lentiviral Particles: sc-42010-V as alternate gene silencing products.

For independent verification of PSD-95 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42010A, sc-42010B and sc-42010C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 µl of RNase-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PSD-95 siRNA (h) is recommended for the inhibition of PSD-95 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

PSD-95 (7E3): sc-32290 is recommended as a control antibody for monitoring of PSD-95 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 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) 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PSD-95 gene expression knockdown using RT-PCR Primer: PSD-95 (h)-PR: sc-42010-PR (20 µl, 596 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.