

SAPAP1 siRNA (h): sc-41997

BACKGROUND

Members of the postsynaptic density-95 (PSD-95)/SAP90 family of membrane-associated guanylate kinase (MAGUK) proteins function as multimodular scaffolds that organize protein-signaling complexes at neuronal synapses. PSD-95/SAP90 binds guanylate kinase-associated protein (GKAP), also designated GK domain-binding protein, DAP-1-a, DAP-1-b, PSD-95 binding protein, PSD-95/SAP90 associated protein, or SAPAP, through the guanylate kinase domain. GKAP is expressed widely in neurons of the cortex and hippocampus and in the Purkinje and granule cells of the cerebellum. GKAP is localized specifically in the PSD of glutamatergic synapses, consistent with its direct interaction with PSD-95 family proteins.

REFERENCES

1. Naisbitt, S., Kim, E., Weinberg, R.J., Rao, A., Yang, F.C., Craig, A.M. and Sheng, M. 1997. Characterization of guanylate kinase-associated protein, a postsynaptic density protein at excitatory synapses that interacts directly with postsynaptic density 95/synapse-associated protein 90. *J. Neurosci.* 17: 5687-5696.
2. Kim, E., Naisbitt, S., Hsueh, Y.P., Rao, A., Rothschild, A., Craig, A.M. and Sheng, M. 1997. GKAP, a novel synaptic protein that interacts with the guanylate kinase-like domain of the PSD-95/SAP90 family of channel clustering molecules. *J. Cell Biol.* 136: 669-678.
3. Deguchi, M., Hata, Y., Takeuchi, M., Ide, N., Hirao, K., Yao, I., Irie, M., Toyoda, A. and Takai, Y. 1998. BEGAIN (brain-enriched guanylate kinase-associated protein), a novel neuronal PSD-95/SAP90-binding protein. *J. Biol. Chem.* 273: 26269-26272.
4. Yamada, Y., Chochi, Y., Ko, J.A., Sobue, K. and Inui, M. 1999. Activation of channel activity of the NMDA receptor-PSD-95 complex by guanylate kinase-associated protein (GKAP). *FEBS Lett.* 458: 295-298.
5. Passafaro, M., Sala, C., Niethammer, M. and Sheng, M. 1999. Microtubule binding by CRIPT and its potential role in the synaptic clustering of PSD-95. *Nat. Neurosci.* 2: 1063-1069.
6. Shin, H., Hsueh, Y.P., Yang, F.C., Kim, E. and Sheng, M. 2000. An intramolecular interaction between Src homology 3 domain and guanylate kinase-like domain required for channel clustering by postsynaptic density-95/SAP90. *J. Neurosci.* 20: 3580-3587.

CHROMOSOMAL LOCATION

Genetic locus: DLGAP1 (human) mapping to 18p11.31.

PRODUCT

SAPAP1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SAPAP1 shRNA Plasmid (h): sc-41997-SH and SAPAP1 shRNA (h) Lentiviral Particles: sc-41997-V as alternate gene silencing products.

For independent verification of SAPAP1 (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-41997A, sc-41997B and sc-41997C.

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

APPLICATIONS

SAPAP1 siRNA (h) is recommended for the inhibition of SAPAP1 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 μ M in 66 μ l. 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

SAPAP1 (21): sc-136524 is recommended as a control antibody for monitoring of SAPAP1 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 SAPAP1 gene expression knockdown using RT-PCR Primer: SAPAP1 (h)-PR: sc-41997-PR (20 μ l). 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.