

E6TP1 siRNA (h): sc-42180

BACKGROUND

The Rap family of small GTPases is closely related to Ras and may function as an antagonist to the Ras signaling pathway by trapping Ras effectors in an inactive complex. Similar to other guanine-binding proteins (such as the heterotrimeric G proteins), the Ras proteins cycle between an active guanosine-triphosphate (GTP) bound form and an inactive, guanosine-diphosphate (GDP) bound form. The weak intrinsic GTPase activity of Ras proteins is greatly enhanced by the action of GTPase-activating proteins (GAPs). E6TP1, also known as signal-induced proliferation-associated 1 like 1 (SIPA1L1) and spine-associated RapGAP (SPAR) is a Rap-specific GTPase-activating protein (RapGAP) that interacts with the guanylate kinase-like domain of post-synaptic density protein-95 (PSD-95) and forms a complex with PSD-95 and with N-methyl-D-aspartate (NMDA) receptors in the brain. In heterologous neurons, E6TP1 reorganizes the Actin cytoskeleton and recruits PSD-95 to F-Actin. In hippocampal neurons, E6TP1 localizes to dendritic spines and causes enlargement of spine heads, many of which adopt an irregular appearance. Transient expression of E6TP1 in HeLa cells localizes to the cortical cytoskeleton and induces rounding up of the cells.

REFERENCES

1. Bos, J. 1998. All in the family? New insights and questions regarding interconnectivity of Ras, Rap1, and Ral. *EMBO J.* 17: 6776-6782.
2. Online Mendelian Inheritance in Man, OMIM™. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 139150. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Zwartkruis, F., et al. 1999. Ras and Rap1: two highly related small GTPases with distinct function. *Exp. Cell Res.* 253: 157-165.
4. Tsukamoto, N., et al. 1999. Rap1 GTPase-activating protein SPA-1 negatively regulates cell adhesion. *J. Biol. Chem.* 274: 18463-18469.
5. Pak, D., et al. 2001. Regulation of dendritic spine morphology by SPAR, a PSD-95 associated RapGAP. *Neuron* 31: 169-171.

CHROMOSOMAL LOCATION

Genetic locus: SIPA1L1 (human) mapping to 14q24.2.

PRODUCT

E6TP1 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 E6TP1 shRNA Plasmid (h): sc-42180-SH and E6TP1 shRNA (h) Lentiviral Particles: sc-42180-V as alternate gene silencing products.

For independent verification of E6TP1 (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-42180A, sc-42180B and sc-42180C.

PROTOCOLS

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

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

E6TP1 siRNA (h) is recommended for the inhibition of E6TP1 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor E6TP1 gene expression knockdown using RT-PCR Primer: E6TP1 (h)-PR: sc-42180-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.