

DEF6 siRNA (h): sc-105283

BACKGROUND

DEF6 (differentially expressed in FDCP 6), also known as IBP, is a 631 amino acid protein that is widely expressed in T and B cells of the immune system. Localized to the nucleus, cytoplasm and cell membrane, DEF6 is a phosphatidylinositol 3,4,5-trisphosphate-dependent guanine nucleotide exchange factor (GEF) that functions to activate Rho GTPases such as Rac 1, Cdc42 and Rho A. Via its ability to activate various Rho GTPases, DEF6 can regulate cell shape, movement and polarity and, through its interaction with T helper cells, DEF6 can also help mediate the immune response. DEF6 contains one PH (pleckstrin homology) domain which is required for binding to phosphatidylinositol 3,4,5-trisphosphate and subsequent recruitment to the plasma membrane.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610094. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Gupta, S., et al. 2003. T cell receptor engagement leads to the recruitment of IBP, a novel guanine nucleotide exchange factor, to the immunological synapse. *J. Biol. Chem.* 278: 43541-43549.
3. Gupta, S., et al. 2003. Molecular cloning of IBP, a SWAP-70 homologous GEF, which is highly expressed in the immune system. *Hum. Immunol.* 64: 389-401.
4. Tanaka, Y., et al. 2003. SWAP-70-like adapter of T cells, an adapter protein that regulates early TCR-initiated signaling in Th2 lineage cells. *Immunity* 18: 403-414.
5. Mavrakis, K.J., et al. 2004. DEF6, a novel PH-DH-like domain protein, is an upstream activator of the Rho GTPases Rac1, Cdc42, and RhoA. *Exp. Cell Res.* 294: 335-344.
6. Samson, T., et al. 2007. Def-6, a guanine nucleotide exchange factor for Rac1, interacts with the skeletal muscle integrin chain $\alpha 7A$ and influences myoblast differentiation. *J. Biol. Chem.* 282: 15730-15742.

CHROMOSOMAL LOCATION

Genetic locus: DEF6 (human) mapping to 6p21.31.

PRODUCT

DEF6 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 DEF6 shRNA Plasmid (h): sc-105283-SH and DEF6 shRNA (h) Lentiviral Particles: sc-105283-V as alternate gene silencing products.

For independent verification of DEF6 (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-105283A, sc-105283B and sc-105283C.

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

DEF6 siRNA (h) is recommended for the inhibition of DEF6 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

DEF6 (SW-9): sc-81992 is recommended as a control antibody for monitoring of DEF6 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 DEF6 gene expression knockdown using RT-PCR Primer: DEF6 (h)-PR: sc-105283-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.