SANTA CRUZ BIOTECHNOLOGY, INC.

DDEF1 siRNA (h): sc-62196

BACKGROUND

DDEF1 (development and differentiation enhancing factor 1), also known as ASAP1, AMAP1 or PAG2, is an ADP ribosylation factor (ARF)-GTPase activating protein (GAP) that interacts with various signal transduction proteins. Localized to the cytoplasm and to newly formed focal complexes at the cell periphery, DDEF1 coordinates with proteins such as ARF1, ARF5, ARF6 and SRK (ZAP-70) to influence growth and differentiation events. Through its interactions with these proteins, DDEF1 plays a key role in cell motility and regulation of Actin cytoskeletal remodeling, as well as in differentiation of adipocytes and fibroblasts. DDEF1 contains two ANK repeats, one ARF-GAP domain, one SH3 domain and one PH domain which is essential in the phosphoinositide-dependent regulation of ARFs. Overexpression of DDEF1 is thought to block the invasion and metastasis of breast cancer and high-grade uveal melanomas, suggesting a possible role as a therapeutic target and diagnostic marker for certain cancers.

REFERENCES

1. Furman, C., et al. 2002. DEF-1/ASAP1 is a GTPase-activating protein (GAP) for ARF1 that enhances cell motility through a GAP-dependent mechanism. J. Biol. Chem. 277: 7962-7969.
2. Onodera, Y., et al. 2005. Expression of AMAP1, an ArfGAP, provides novel targets to inhibit breast cancer invasive activities. EMBO J. 24: 963-973.
3. Ehlers, J.P., et al. 2005. DDEF1 is located in an amplified region of chromosome 8q and is overexpressed in uveal melanoma. Clin. Cancer Res. 11: 3609-3613.
4. Che, M.M., et al. 2005. Regulation of ASAP1 by phospholipids is dependent on the interface between the PH and Arf GAP domains. Cell. Signal. 17: 1276-1288.
5. Luo, R., et al. 2005. Mutational analysis of the Arf1*GTP/Arf GAP interface reveals an Arf1 mutant that selectively affects the Arf GAP ASAP1. Curr. Biol. 15: 2164-2169.
6. Nie, Z., et al. 2006. A BAR domain in the N terminus of the Arf GAP ASAP1 affects membrane structure and trafficking of epidermal growth factor receptor. Curr. Biol. 16: 130-139.

CHROMOSOMAL LOCATION

Genetic locus: ASAP1 (human) mapping to 8q24.21.

PRODUCT

DDEF1 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 DDEF1 shRNA Plasmid (h): sc-62196-SH and DDEF1 shRNA (h) Lentiviral Particles: sc-62196-V as alternate gene silencing products.

For independent verification of DDEF1 (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-62196A, sc-62196B and sc-62196C.

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

DDEF1 siRNA (h) is recommended for the inhibition of DDEF1 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

DDEF1 (B-10): sc-374410 is recommended as a control antibody for monitoring of DDEF1 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 DDEF1 gene expression knockdown using RT-PCR Primer: DDEF1 (h)-PR: sc-62196-PR (20 µl). 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.