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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

SANTA CRUZ BIOTECHNOLOGY, INC.

DDEF2 siRNA (h): sc-41694

BACKGROUND

DDEF2 (ADP ribosylation factor [ARF]-GTPase-activating protein [GAP] containing SH3, ANK repeats, and PH domain, PAP, PAG2, AMAP1, ZG14P, centaurin β4) is a phospholipid-dependent ADP-ribosylation factor (ARF) GTPase-activating protein (ARF-GAP) that binds to protein-tyrosine kinases Src and focal adhesion kinase. ARF family GTP-binding proteins are regulators of membrane traffic and cytoskeletal organization. Modulation of ARF activity by DDEF2 is important for the regulation of focal adhesion assembly and/or organization by influencing the mechanisms responsible for the recruitment and organization of focal adhesion proteins paxillin and FAK. In spreading platelets, most endogenous DDEF2 is localized at peripheral focal adhesions. Pyk2 directly phosphorylates DDEF2 on Tyrosine 308 and 782, and this event affects the phosphoinositide binding profile of DDEF2. DDEF2 is phosphorylated on tyrosine residues in cells expressing activated Src and tyrosine phosphorylation depends on a proline-rich class II Src SH3 binding site.

REFERENCES

1. Brown, M.T., et al. 1998. ASAP1, a phospholipid-dependent arf GTPase-activating protein that associates with and is phosphorylated by Src. Mol. Cell. Biol. 18: 7038-7051.
2. Randazzo, P.A., et al. 2000. The Arf GTPase-activating protein ASAP1 regulates the actin cytoskeleton. Proc. Natl. Acad. Sci. USA 97: 4011-4016.
3. Kam, J.L., et al. 2000. Phosphoinositide-dependent activation of the ADP-ribosylation factor GTPase-activating protein ASAP1. Evidence for the pleckstrin homology domain functioning as an allosteric site. J. Biol. Chem. 275: 9653-9663.
4. 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.
5. Liu, Y., et al. 2002. The association of ASAP1, an ADP ribosylation factor-GTPase activating protein, with focal adhesion kinase contributes to the process of focal adhesion assembly. Mol. Biol. Cell 13: 2147-2156.
6. Oda, A., et al. 2003. CrkL directs ASAP1 to peripheral focal adhesions. J. Biol. Chem. 278: 6456-6460.

CHROMOSOMAL LOCATION

Genetic locus: ASAP2 (human) mapping to 2p25.1.

PRODUCT

DDEF2 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 DDEF2 shRNA Plasmid (h): sc-41694-SH and DDEF2 shRNA (h) Lentiviral Particles: sc-41694-V as alternate gene silencing products.

For independent verification of DDEF2 (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-41694A, sc-41694B and sc-41694C.

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

DDEF2 siRNA (h) is recommended for the inhibition of DDEF2 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

DDEF2 (C-9): sc-374323 is recommended as a control antibody for monitoring of DDEF2 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 DDEF2 gene expression knockdown using RT-PCR Primer: DDEF2 (h)-PR: sc-41694-PR (20 µl, 560 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.