

ADF siRNA (h): sc-43200

BACKGROUND

Actin-depolymerizing factor (ADF), also known as destrin, is a member of the ADF/Cofilin/destrin superfamily that has the ability to rapidly depolymerize F-Actin in a stoichiometric manner. The Actin-depolymerizing activity of ADF is reversibly controlled by changes in KCl concentration but is insensitive to calcium concentration. ADF depolymerizes F-Actin by interacting directly with F-Actin protomers. ADF shares 71% sequence homology with Cofilin, however the two proteins differ in their interaction with Actin. The difference in the function of ADF and Cofilin results from the subtle difference in their amino acid sequence rather than possible differences in posttranslational modifications. As a result of different cleavage sites on ADF and Cofilin, the proteins differ in their overall tertiary folds. Sensitivity to polyphosphoinositides may be a common feature *in vitro* among Actin-binding proteins such as ADF and Cofilin that can bind to G-Actin and regulate the state of Actin polymerization. ADF and Cofilin are Actin-depolymerizing proteins whose activities are possibly regulated by their phosphorylation/dephosphorylation.

REFERENCES

1. Nishida, E., Muneyuki, E., Maekawa, S., Ohta, Y. and Sakai, H. 1985. An Actin-depolymerizing protein (destrin) from porcine kidney. Its action on F-Actin containing or lacking Tropomyosin. *Biochemistry* 24: 6624-6630.
2. Moriyama, K., Nishida, E., Yonezawa, N., Sakai, H., Matsumoto, S., Iida, K. and Yahara, I. 1990. Destrin, a mammalian Actin-depolymerizing protein, is closely related to Cofilin. Cloning and expression of porcine brain destrin cDNA. *J. Biol. Chem.* 265: 5768-5773.
3. Yonezawa, N., Nishida, E., Iida, K., Yahara, I. and Sakai, H. 1990. Inhibition of the interactions of Cofilin, destrin and deoxyribonuclease I with Actin by phosphoinositides. *J. Biol. Chem.* 265: 8382-8386.
4. Kanamori, T., Hayakawa, T., Suzuki, M. and Titani, K. 1995. Identification of two 17 kDa rat parotid gland phosphoproteins, subjects for dephosphorylation upon β -adrenergic stimulation, as destrin- and Cofilin-like proteins. *J. Biol. Chem.* 270: 8061-8067.
5. Arima, K., Imanaka, M., Okuzono, S., Kazuta, Y. and Kotani, S. 1998. Evidence for structural differences between the two highly homologous Actin-regulatory proteins, destrin and Cofilin. *Biosci. Biotechnol. Biochem.* 62: 215-220.

CHROMOSOMAL LOCATION

Genetic locus: DSTN (human) mapping to 20p12.1.

PRODUCT

ADF 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 ADF shRNA Plasmid (h): sc-43200-SH and ADF shRNA (h) Lentiviral Particles: sc-43200-V as alternate gene silencing products.

For independent verification of ADF (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-43200A, sc-43200B and sc-43200C.

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

ADF siRNA (h) is recommended for the inhibition of ADF 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 ADF gene expression knockdown using RT-PCR Primer: ADF (h)-PR: sc-43200-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Moore, A.S., Coscia, S.M., Simpson, C.L., Ortega, F.E., Wait, E.C., Hedderston, J.M., Nirschl, J.J., Obara, C.J., Guedes-Dias, P., Boecker, C.A., Chew, T.L., Theriot, J.A., Lippincott-Schwartz, J. and Holzbaur, E.L.F. 2021. Actin cables and comet tails organize mitochondrial networks in mitosis. *Nature* 591: 659-664.

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.