

MACF1 siRNA (h): sc-75724

BACKGROUND

MACF1 (microtubule-Actin cross-linking factor 1) is a 5,327 amino acid protein that is encoded by the human gene MACF1. MACF1 belongs to the plakin or cytolinker family and contains one Actin-binding domain, 2 CH (calponin-homology) domains, 2 EF-hand domains, one SH3 domain and 37 spectrin repeats. MACF1 is an F-Actin-binding protein which may play a role in cross-linking Actin to other cytoskeletal proteins and also binds to microtubules. The spectrin repeats, an important feature found in many proteins involved in cytoskeletal structure, form a three helix bundle with the second helix (with proline interrupts in some sequences). MACF1 is a cytoplasmic protein expressed mainly in lung, brain, spinal cord, skeletal and cardiac muscle, and skin.

REFERENCES

1. Kakinuma, T., et al. 2004. Interaction between p230 and MACF1 is associated with transport of a glycosyl phosphatidyl inositol-anchored protein from the Golgi to the cell periphery. *Exp. Cell Res.* 298: 388-398.
2. Lin, C.M., et al. 2005. Microtubule Actin crosslinking factor 1b: a novel plakin that localizes to the Golgi complex. *J. Cell Sci.* 118: 3727-3738.
3. Chen, H.J., et al. 2006. The role of microtubule Actin cross-linking factor 1 (MACF1) in the Wnt signaling pathway. *Genes Dev.* 20: 1933-1945.
4. Trinidad, J.C., et al. 2006. Comprehensive identification of phosphorylation sites in postsynaptic density preparations. *Mol. Cell. Proteomics* 5: 914-922.
5. Dai, J., et al. 2007. Protein phosphorylation and expression profiling by yin-yang multidimensional liquid chromatography (yin-yang MDLC) mass spectrometry. *J. Proteome Res.* 6: 250-262.
6. Munton, R.P., et al. 2007. Qualitative and quantitative analyses of protein phosphorylation in naive and stimulated mouse synaptosomal preparations. *Mol. Cell. Proteomics* 6: 283-293.
7. Villén, J., et al. 2007. Large-scale phosphorylation analysis of mouse liver. *Proc. Natl. Acad. Sci. USA* 104: 1488-1493.
8. LocusLink Report (LocusID: 23499). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: MACF1 (human) mapping to 1p34.3.

PRODUCT

MACF1 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 MACF1 shRNA Plasmid (h): sc-75724-SH and MACF1 shRNA (h) Lentiviral Particles: sc-75724-V as alternate gene silencing products.

For independent verification of MACF1 (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-75724A, sc-75724B and sc-75724C.

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

MACF1 siRNA (h) is recommended for the inhibition of MACF1 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

MACF1-4 (D-10): sc-377534 is recommended as a control antibody for monitoring of MACF1 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 MACF1 gene expression knockdown using RT-PCR Primer: MACF1 (h)-PR: sc-75724-PR (20 μ l, 542 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.