SANTA CRUZ BIOTECHNOLOGY, INC.

MAPBPIP siRNA (h): sc-88091

BACKGROUND

MP1 (MEK partner 1) functions as a scaffolding protein in the mitogen activated protein (MAP) kinase signaling pathway. Growth factor induced MAP kinase activation is selectively mediated by the extracellular signal-regulated kinase (ERK) cascade. MAPBPIP (mitogen-activated protein-binding protein-interacting protein), also known as p14 and late endosomal/lysosomal MP1-interacting protein, functions as an adaptor protein augmenting the regulation of the MAP kinase cascade. Partner proteins MAPBPIP and MP1 are structurally almost identical each with a five-stranded β-sheet flanked between a two-helix and one-helix layer. MAPBPIP compels the recruitment of MP1 to late endosomes where they form a very stable heterodimeric complex required for ERK activation on endosomes. Knockdown of the individual proteins in the MP1/MAPBPIP complex resulted in decreased expression of the partner proteins which implies greater stability of the heterodimeric complex than either MP1 or MAPBPIP individually. Early research suggests the MP1-MAPBPIP-MEK-1 signaling complex may be critical in the regulation of tissue homeostasis.

REFERENCES

1. Wunderlich, W., et al. 2001. A novel 14 kDa protein interacts with the mitogen-activated protein kinase scaffold MP1 on a late endosomal/lysosomal compartment. J. Cell Biol. 152: 765-776.
2. Teis, D., et al. 2002. Localization of the MP1-MAPK scaffold complex to endosomes is mediated by p14 and required for signal transduction. Dev. Cell 3: 803-814.
3. Kurzbauer, R., et al. 2004. Crystal structure of the p14/MP1 scaffolding complex: how a twin couple attaches mitogen-activated protein kinase signaling to late endosomes. Proc. Natl. Acad. Sci. USA 101: 10984-10989.
4. Lunin, V.V., et al. 2004. The structure of the MAPK scaffold, MP1, bound to its partner, p14. A complex with a critical role in endosomal map kinase signaling. J. Biol. Chem. 279: 23422-23430.
5. Pullikuth, A., et al. 2005. The MEK-1 scaffolding protein MP1 regulates cell spreading by integrating PAK1 and Rho signals. Mol. Cell. Biol. 25: 5119-5133.

CHROMOSOMAL LOCATION

Genetic locus: ROBLD3 (human) mapping to 1q22.

PRODUCT

MAPBPIP siRNA (h) is a pool of 2 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 MAPBPIP shRNA Plasmid (h): sc-88091-SH and MAPBPIP shRNA (h) Lentiviral Particles: sc-88091-V as alternate gene silencing products.

For independent verification of MAPBPIP (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-88091A and sc-88091B.

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

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

MAPBPIP (B-5): sc-515708 is recommended as a control antibody for monitoring of MAPBPIP 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 MAPBPIP gene expression knockdown using RT-PCR Primer: MAPBPIP (h)-PR: sc-88091-PR (20 µl, 406 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.