

MKLP-1 siRNA (r): sc-270014

BACKGROUND

The monoclonal antibody CHO1 detects a spindle antigen required for mitotic progression. Screening a HeLa cell cDNA expression library with this antibody has been shown to yield a cDNA predicted to encode a protein significantly related within its amino terminal half to the motor ends of members of the kinesin superfamily. Since this similarity does not extend further, it has been suggested that the CHO1 antigen, now designated MKLP-1 (mitotic kinesin-like protein-1), represents a novel kinesin. Sequence analysis has also been shown to predict that MKLP-1 possesses features typical of nuclear proteins. Immunocytological studies have demonstrated that MKLP-1 moves from the nucleus early in mitosis and then to the midbody after cytokinesis. MKLP-1 has been shown to bundle antiparallel microtubules *in vitro* and to move them at rates comparable to spindle elongation *in vivo*. A hamster homolog of MKLP-1, designated CHO1 antigen, has also been isolated. Although apparently functionally equivalent with respect to microtubule bundling activity, there are significant differences between the human and hamster proteins at their C-termini, possibly due to alternative splicing or the presence of more than one MKLP-1 locus.

REFERENCES

1. Sellitto, C., et al. 1988. Distribution of a matrix component of the midbody during the cell cycle in Chinese hamster ovary cells. *J. Cell Biol.* 106: 431-439.
2. Roberts, B. 1989. Nuclear location signal-mediated protein transport. *Biochim. Biophys. Acta* 1008: 263-280.
3. Nislow, C., et al. 1990. A monoclonal antibody to a mitotic microtubule-associated protein blocks mitotic progression. *J. Cell Biol.* 111: 511-522.
4. Kuriyama, R., et al. 1992. Molecular components of the mitotic spindle. *Bioessays* 14: 81-88.
5. Nislow, C., et al. 1992. A plus-end-directed motor enzyme that moves antiparallel microtubules *in vitro* localizes to the interzone of mitotic spindles. *Nature* 359: 543-547.
6. Goldstein, L.S. 1993. With apologies to Scheherazade: tails of 1001 kinesin motors. *Annu. Rev. Genet.* 27: 319-351.

CHROMOSOMAL LOCATION

Genetic locus: Kif23 (rat) mapping to 8q24.

PRODUCT

MKLP-1 siRNA (r) 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 MKLP-1 shRNA Plasmid (r): sc-270014-SH and MKLP-1 shRNA (r) Lentiviral Particles: sc-270014-V as alternate gene silencing products.

For independent verification of MKLP-1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270014A, sc-270014B and sc-270014C.

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

MKLP-1 siRNA (r) is recommended for the inhibition of MKLP-1 expression in rat 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

MKLP-1 (C-12): sc-390113 is recommended as a control antibody for monitoring of MKLP-1 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 MKLP-1 gene expression knockdown using RT-PCR Primer: MKLP-1 (r)-PR: sc-270014-PR (20 μ l). 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.