

MCM2 siRNA (h): sc-35879

BACKGROUND

The mini-chromosome maintenance (MCM) family of proteins, including MCM2, MCM3, MCM4 (Cdc21), MCM5 (Cdc46), MCM6 (Mis5) and MCM7 (Cdc47), are regulators of DNA replication that act to ensure replication occurs only once in the cell cycle. Expression of MCM proteins increases during cell growth, peaking at G₁ to S phase. The MCM proteins each contain an ATP-binding motif, which is predicted to mediate ATP-dependent opening of double-stranded DNA. MCM proteins are regulated by E2F transcription factors, which induce MCM expression, and by protein kinases, which interact with MCM proteins to maintain the postreplicative state of the cell. MCM2/MCM4 complexes function as substrates for Cdc2/cyclin B *in vitro*. Cleavage of MCM3, which can be prevented by caspase inhibitors, results in the inactivation during apoptosis of the MCM complex, which is composed of, at least, MCM2-6. A complex composed of MCM4, MCM6 and MCM7 has been shown to be involved in DNA helicase activity, and MCM5 is involved in IFN- γ -induced Stat1 α transcription activation.

REFERENCES

1. Koonin, E.V. 1993. A common set of conserved motifs in a vast variety of putative nucleic acid-dependent ATPases including MCM proteins involved in the initiation of eukaryotic DNA replication. *Nucleic Acids Res.* 21: 2541-2547.
2. Ishimi, Y. 1997. A DNA helicase activity is associated with an MCM4, -6, and -7 protein complex. *J. Biol. Chem.* 272: 24508-24513.
3. Leone, G., et al. 1998. E2F3 activity is regulated during the cell cycle and is required for the induction of S phase. *Genes Dev.* 12: 2120-2130.
4. Coverley, D., et al. 1998. Protein kinase inhibition in G₂ causes mammalian MCM proteins to reassociate with chromatin and restores ability to replicate. *Exp. Cell Res.* 238: 63-69.
5. Fujita, M., et al. 1998. Cell cycle- and chromatin binding state-dependent phosphorylation of human MCM heterohexameric complexes. A role for cdc2 kinase. *J. Biol. Chem.* 273: 17095-17101.
6. Schwab, B.L., et al. 1998. Selective proteolysis of the nuclear replication factor MCM3 in apoptosis. *Exp. Cell Res.* 238: 415-421.

CHROMOSOMAL LOCATION

Genetic locus: MCM2 (human) mapping to 3q21.3.

PRODUCT

MCM2 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 MCM2 shRNA Plasmid (h): sc-35879-SH and MCM2 shRNA (h) Lentiviral Particles: sc-35879-V as alternate gene silencing products.

For independent verification of MCM2 (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-35879A, sc-35879B and sc-35879C.

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

MCM2 siRNA (h) is recommended for the inhibition of MCM2 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

MCM2 (E-8): sc-373702 is recommended as a control antibody for monitoring of MCM2 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 MarkerTM 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 MCM2 gene expression knockdown using RT-PCR Primer: MCM2 (h)-PR: sc-35879-PR (20 μ l, 413 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.