

Emi1 siRNA (m): sc-44344

BACKGROUND

Emi1 (for early mitotic inhibitor) regulates mitosis by inhibiting the anaphase promoting complex/cyclosome (APC). Emi1 is a conserved F box protein containing a zinc binding region essential for APC inhibition. The Emi1 protein functions to promote cyclin A accumulation and S phase entry in somatic cells by inhibiting the APC complex. At the G₁-S transition, Emi1 is transcriptionally induced by the E2F transcription factor. Emi1 overexpression accelerates S phase entry and can override a G₁ block caused by overexpression of Cdh1 or the E2F-inhibitor p105 retinoblastoma protein (pRb). Depleting cells of Emi1 through RNA interference prevents accumulation of cyclin A and inhibits S phase entry. Emi1 is required to arrest unfertilized eggs at metaphase of meiosis II and may be the long-sought mediator of CSF activity. Human Emi1 is similar to *Xenopus laevis* Emi1, which inhibits the APC (Cdc20) ubiquitination complex to allow accumulation of cyclin B.

REFERENCES

1. Cenciarelli, C., et al. 1999. Identification of a family of human F-box proteins. *Curr. Biol.* 9: 1177-1179.
2. Chiaur, D.S., et al. 2000. Five human genes encoding F-box proteins: chromosome mapping and analysis in human tumors. *Cytogenet. Cell Genet.* 88: 255-258.
3. Reimann, J.D., et al. 2001. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the anaphase promoting complex. *Cell* 105: 645-655.
4. Reimann, J.D., et al. 2002. Emi1 is required for cytostatic factor arrest in vertebrate eggs. *Nature* 416: 850-854.
5. Jin, J., et al. 2004. Systematic analysis and nomenclature of mammalian F-box proteins. *Genes Dev.* 18: 2573-2580.
6. Eldridge, A.G., et al. 2006. The *evi5* oncogene regulates cyclin accumulation by stabilizing the anaphase-promoting complex inhibitor *emi1*. *Cell* 124: 367-380.
7. Lee, H., et al. 2006. Mouse *emi1* has an essential function in mitotic progression during early embryogenesis. *Mol. Cell. Biol.* 26: 5373-5381.
8. Lehman, N.L., et al. 2006. Overexpression of the anaphase promoting complex/cyclosome inhibitor Emi1 leads to tetraploidy and genomic instability of p53-deficient cells. *Cell Cycle* 5: 1569-1573.

CHROMOSOMAL LOCATION

Genetic locus: Fbxo5 (mouse) mapping to 10 A1.

PRODUCT

Emi1 siRNA (m) 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 Emi1 shRNA Plasmid (m): sc-44344-SH and Emi1 shRNA (m) Lentiviral Particles: sc-44344-V as alternate gene silencing products.

For independent verification of Emi1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44344A, sc-44344B and sc-44344C.

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

Emi1 siRNA (m) is recommended for the inhibition of Emi1 expression in mouse 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

Emi1 (B-3): sc-365212 is recommended as a control antibody for monitoring of Emi1 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 Emi1 gene expression knockdown using RT-PCR Primer: Emi1 (m)-PR: sc-44344-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.