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# Emi1 shRNA (m) Lentiviral Particles: sc-44344-V

## 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 G<sub>1</sub> 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

- Cenciarelli, C., et al. 1999. Identification of a family of human F-box proteins. *Curr. Biol.* 9: 1177-1179.
- 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.
- 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.
- Reimann, J.D., et al. 2002. Emi1 is required for cytostatic factor arrest in vertebrate eggs. *Nature* 416: 850-854.
- Jin, J., et al. 2004. Systematic analysis and nomenclature of mammalian F-box proteins. *Genes Dev.* 18: 2573-2580.

## CHROMOSOMAL LOCATION

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

## PRODUCT

Emi1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Emi1 siRNA (m): sc-44344 and Emi1 shRNA Plasmid (m): sc-44344-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

Emi1 shRNA (m) Lentiviral Particles is recommended for the inhibition of Emi1 expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

Emi1 (P-20): sc-50929 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.