

Emi2 siRNA (h): sc-77268

BACKGROUND

F-box proteins are critical components of the SCF (Skp1-CUL-1-F-box protein) type E3 ubiquitin ligase complex and are involved in substrate recognition and recruitment for ubiquitination. They are members of a larger family of proteins that are involved in the regulation of a wide variety of cellular processes (including the cell cycle, immune responses, signaling cascades and developmental events) through the targeting of proteins, such as cyclins, cyclin-dependent kinase inhibitors, I κ B- α and β -catenin, for proteasomal degradation. Emi2 (endogenous meiotic inhibitor 2), also known as FBXO43 (F-box only protein 43) or ERP1, is a 708 amino acid protein that contains one F-box domain and one IBR-type zinc finger. Playing an important role in protein modification, Emi2 is required for the establishment and maintenance of oocyte arrest at the second meiotic metaphase, an event that is crucial for fertilization. Specifically, Emi2 is thought to induce meiotic arrest by inhibiting the activity of the APC (anaphase-promoting complex), thereby preventing the progression of meiosis. Emi2 is subject to phosphorylation and ubiquitination, both of which promote its degradation by the proteasome.

REFERENCES

1. Jin, J., et al. 2004. Systematic analysis and nomenclature of mammalian F-box proteins. *Genes Dev.* 18: 2573-2580.
2. Tung, J.J., et al. 2005. A role for the anaphase-promoting complex inhibitor Emi2/XErp1, a homolog of early mitotic inhibitor 1, in cytostatic factor arrest of *Xenopus* eggs. *Proc. Natl. Acad. Sci. USA* 102: 4318-4323.
3. Shoji, S., et al. 2006. Mammalian Emi2 mediates cytostatic arrest and transduces the signal for meiotic exit via Cdc20. *EMBO J.* 25: 834-845.
4. Hansen, D.V., et al. 2006. CaMKII and polo-like kinase 1 sequentially phosphorylate the cytostatic factor Emi2/XErp1 to trigger its destruction and meiotic exit. *Proc. Natl. Acad. Sci. USA* 103: 608-613.

CHROMOSOMAL LOCATION

Genetic locus: FBXO43 (human) mapping to 8q22.2.

PRODUCT

Emi2 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 Emi2 shRNA Plasmid (h): sc-77268-SH and Emi2 shRNA (h) Lentiviral Particles: sc-77268-V as alternate gene silencing products.

For independent verification of Emi2 (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-77268A, sc-77268B and sc-77268C.

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.

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

Emi2 siRNA (h) is recommended for the inhibition of Emi2 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

Emi2 (4B6): sc-517089 is recommended as a control antibody for monitoring of Emi2 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 Emi2 gene expression knockdown using RT-PCR Primer: Emi2 (h)-PR: sc-77268-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.