

DREAM siRNA (h): sc-42398

BACKGROUND

DREAM (for DRE-antagonist modulator) is a Ca^{2+} -regulated transcriptional repressor that specifically binds to the downstream regulatory elements (DRE). DRE is a regulatory sequence that silences basal transcription and is localized to the promoter region of the gene encoding human prodynorphin, an opioid peptide involved in memory acquisition and pain. DREAM forms functional homotetramers that are required for the interaction with the DRE. This association is highly influenced by calcium, as an increase in Ca^{2+} directly inhibits DREAM binding and thereby blocks the repressor activity of DREAM. DREAM transcripts are detected in brain, thymus and thyroid gland, and it is expressed as a nuclear protein. DREAM has been shown to inhibit transcription of other proteins containing DRE-like motifs, including the gene encoding for the AP-1 transcription factor c-Fos, suggesting that DREAM may influence a wide variety of cellular genes.

REFERENCES

1. Morgan, J.I., et al. 1986. Role of ion flux in the control of c-Fos expression. *Nature* 322: 552-555.
2. Weisskopf, M.G., et al. 1993. The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibre synapses and modulates long-term potentiation. *Nature* 365: 188.
3. Hurd, Y.L. 1996. Differential messenger RNA expression of prodynorphin and proenkephalin in the human brain. *Neuroscience* 72: 767-783.
4. Carrion, A.M., et al. 1998. Protein kinase A-dependent derepression of the human prodynorphin gene via differential binding to an intragenic silencer element. *Mol. Cell. Biol.* 18: 6921-6929.

CHROMOSOMAL LOCATION

Genetic locus: KCNIP3 (human) mapping to 2q11.1.

PRODUCT

DREAM 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DREAM shRNA Plasmid (h): sc-42398-SH and DREAM shRNA (h) Lentiviral Particles: sc-42398-V as alternate gene silencing products.

For independent verification of DREAM (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-42398A, sc-42398B and sc-42398C.

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 μl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μl of RNase-free water makes a 10 μM solution in a 10 μM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

DREAM siRNA (h) is recommended for the inhibition of DREAM 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 μM in 66 μl . 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

DREAM (A-9): sc-166916 is recommended as a control antibody for monitoring of DREAM 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 DREAM gene expression knockdown using RT-PCR Primer: DREAM (h)-PR: sc-42398-PR (20 μl , 434 bp). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

SELECT PRODUCT CITATIONS

1. Baczyk, D., et al. 2013. DREAM mediated regulation of GCM1 in the human placental trophoblast. *PLoS ONE* 8: e51837.
2. Fontán-Lozano, A., et al. 2016. Impact of transient down-regulation of DREAM in human embryonic stem cell pluripotency: the role of DREAM in the maintenance of hESCs. *Stem Cell Res.* 16: 568-578.

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.