

CRF siRNA (m): sc-39396

BACKGROUND

Individuals suffering from Alzheimer's disease (AD) exhibit dramatic reductions in the content of corticotropin-releasing factor (CRF), increased expression of CRF receptors (CRFRs) and abnormalities in neuronal morphology in affected brain areas. In addition, AD patients show decreased concentrations of CRF in their cerebrospinal fluid, which may contribute to their cognitive impairment. A high affinity CRF binding protein, designated CRF-BP, has been discovered in post-mortem brain samples from AD patients. CRF-BP serves to bind and inactivate CRF, reducing the pool of "free CRF" available to bind CRFRs. Two CRFRs, designated CRF-RI and CRF-RII, have been described and exhibit distinct brain localizations. There are two forms of CRF-RII, referred to as CRF-RII α and CRF-RII β , that result from alternative mRNA splicings. An additional member of the CRF family, urocortin, shares 63% sequence identity with urotensin and 45% sequence identity with CRF. Urocortin specifically binds to and activates CRF-RI and CRF-RII, but binds to CRF-RII more efficiently than CRF, suggesting that it may be the true, high affinity ligand for the CRF receptor type II.

REFERENCES

- Behan, D.P., et al. 1995. Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. *Nature* 378: 284-287.
- Behan, D.P., et al. 1995. Corticotropin releasing factor binding protein (CRF-BP) is expressed in neuronal and astrocytic cells. *Brain Res.* 698: 259-264.
- Behan, D.P., et al. 1995. Corticotropin releasing factor (CRF) binding protein: a novel regulator of CRF and related peptides. *Frontiers Neuroendocrinol.* 16: 362-382.
- Chalmers, D.T., et al. 1995. Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. *J. Neurosci.* 15: 6340-6350.
- Lovenberg, T.W., et al. 1995. CRF2 α and CRF2 β receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. *Endocrinology* 136: 4139-4142.

CHROMOSOMAL LOCATION

Genetic locus: Crh (mouse) mapping to 3 A2.

PRODUCT

CRF 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 CRF shRNA Plasmid (m): sc-39396-SH and CRF shRNA (m) Lentiviral Particles: sc-39396-V as alternate gene silencing products.

For independent verification of CRF (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-39396A, sc-39396B and sc-39396C.

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

CRF siRNA (m) is recommended for the inhibition of CRF 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

CRF (2B11): sc-293187 is recommended as a control antibody for monitoring of CRF 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 CRF gene expression knockdown using RT-PCR Primer: CRF (h)-PR: sc-39395-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.