

FMR1 siRNA (h): sc-36870

BACKGROUND

Fragile X syndrome is the most frequent form of inherited mental retardation and is the result of transcriptional silencing of the FMR1 gene on the X chromosome. The FMR1 gene contains a distinct CpG dinucleotide repeat located in the 5' untranslated region of the gene. In fragile X syndrome this tandem repeat is substantially amplified and subjected to extensive methylation and enhanced transcriptional silencing. The FMR1 protein (or FMRP) is an RNA-binding protein that associates with polyribosomes and is a likely component of a messenger ribonuclear protein (mRNP) particle. It contains several features that are characteristics of RNA-binding proteins, including two hnRNP K homology (KH) domains and an RGG amino acid motif (RGG box). FMR1 localizes to both the nucleus and the cytoplasm and can also interact with two fragile X syndrome related factors, FXR1 and FXR2, which form heterodimers through their N-terminal coiled-coil domains. Since FMR1 contains both a nuclear localization signal and a nuclear export signal it is also implicated in the nucleocytoplasmic transport of mRNAs.

REFERENCES

1. Verkerk, A.J., et al. 1991. Identification of a gene (FMR1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65: 905-914.
2. Pieretti, M., et al. 1991. Absence of expression of the FMR1 gene in fragile X syndrome. *Cell* 66: 817-822.
3. Matunis, M.J., et al. 1992. Characterization and primary structure of the poly(C)-binding heterogeneous nuclear ribonucleoprotein complex K protein. *Mol. Cell. Biol.* 12: 164-171.

CHROMOSOMAL LOCATION

Genetic locus: FMR1 (human) mapping to Xq27.3.

PRODUCT

FMR1 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 FMR1 shRNA Plasmid (h): sc-36870-SH and FMR1 shRNA (h) Lentiviral Particles: sc-36870-V as alternate gene silencing products.

For independent verification of FMR1 (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-36870A, sc-36870B and sc-36870C.

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

FMR1 siRNA (h) is recommended for the inhibition of FMR1 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

FMR1 (148.1): sc-101048 is recommended as a control antibody for monitoring of FMR1 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 MarkerTM 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 FMR1 gene expression knockdown using RT-PCR Primer: FMR1 (h)-PR: sc-36870-PR (20 μ l, 524 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lee, E.K., et al. 2010. hnRNP C promotes APP translation by competing with FMRP for APP mRNA recruitment to P bodies. *Nat. Struct. Mol. Biol.* 17: 732-739.

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.