

Fe65 siRNA (m): sc-41955

BACKGROUND

γ -secretase cleaves the cell surface protein amyloid protein precursor (APP) at the transmembrane region into an extracellular peptide (β -Amyloid) and an intracellular tail fragment. The cytoplasmic tail of APP forms a multimeric complex with Fe65 (also known as APBB1 for APP binding protein family B member 1). Specifically, Fe65 binds the YENPTY sequence in the cytoplasmic tail of APP. Fe65 is a nuclear adaptor protein widely expressed in the brain, including hippocampus and isocortex. In the cell, Fe65 and APP co-localize to the ER and Golgi. The interaction between APP and Fe65 increases the translocation of APP to the cell surface and the subsequent secretion of β -Amyloid. Fe65 and APP localize with Mena, a cell-adhesion protein, and Fe65 regulates APP-dependent changes in cell motility. The gene encoding human Fe65 maps to chromosome 11p15.4.

REFERENCES

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2. Bressler, S.L., et al. 1996. cDNA cloning and chromosome mapping of the human Fe65 gene: interaction of the conserved cytoplasmic domains of the human β -Amyloid precursor protein and its homologues with the mouse Fe65 protein. *Hum. Mol. Genet.* 5: 1589-1598.
3. Borg, J.P., et al. 1996. The phosphotyrosine interaction domains of X11 and Fe65 bind to distinct sites on the YENPTY motif of amyloid precursor protein. *Mol. Cell. Biol.* 16: 6229-6241.
4. Guenette, S.Y., et al. 1999. hFe65L influences amyloid precursor protein maturation and secretion. *J. Neurochem.* 73: 985-993.
5. Sabo, S.L., et al. 1999. Regulation of β -Amyloid secretion by Fe65, an amyloid protein precursor-binding protein. *J. Biol. Chem.* 274: 7952-7957.
6. Ando, K., et al. 2001. Phosphorylation-dependent regulation of the interaction of amyloid precursor protein with Fe65 affects the production of β -Amyloid. *J. Biol. Chem.* 276: 40353-40361.
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CHROMOSOMAL LOCATION

Genetic locus: Apbb1 (mouse) mapping to 7 E3.

PRODUCT

Fe65 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 Fe65 shRNA Plasmid (h): sc-41954-SH and Fe65 shRNA (h) Lentiviral Particles: sc-41954-V as alternate gene silencing products.

For independent verification of Fe65 (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-41954A, sc-41954B and sc-41954C.

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

Fe65 siRNA (m) is recommended for the inhibition of Fe65 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

Fe65 (F-6): sc-398389 is recommended as a control antibody for monitoring of Fe65 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 Fe65 gene expression knockdown using RT-PCR Primer: Fe65 (m)-PR: sc-41955-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.