SANTA CRUZ BIOTECHNOLOGY, INC.

α-2M siRNA (h): sc-40297

BACKGROUND

α-2-Macroglobulin (α-2M) is a homotetrameric serum protein consisting of four identical subunits that form dimers through disulfide bonds. Initially, α-2M was characterized as a pan-proteinase inhibitor that was able to "bait" proteinases into cleaving specific peptide sequences on α-2M. This interaction induces a conformational change in α-2M, thus enabling it to "trap" the proteinase and further inhibit its activity. Subsequently, α-2M has been shown to function as a carrier protein and regulator of cytokines during inflammation. Circulating transforming growth factor β (TGFβ) in serum is primarily bound to α-2M, which renders TGFβ inactive. α-2M also binds to IL-6 and thereby increases the concentration of IL-6 near lymphocytes, hepatocytes and stem cells involved in mediating the inflammatory cascade. Mutations and deletions in the gene encoding α-2M are associated with an increased incidence of Alzheimer's Disease (AD), which is consistent with the role of α-2M in mediating the clearance and degradation of A β, the major component of β-Amyloid deposits accumulated during AD.

REFERENCES

1. Barrett, A.J., et al. 1973. The interaction of α-2-Macroglobulin with proteinases. Characteristics and specificity of the reaction, and a hypothesis concerning its molecular mechanism. Biochem. J. 133: 709-724.
2. Tsuchiya, Y., et al. 1987. Sequence analysis of the putative regulatory region of rat α-2-Macroglobulin gene. Gene 57: 73-80.
3. Borth, W., et al. 1990. Binding of IL-1β to α-Macroglobulins and release by Thioredoxin. J. Immunol. 145: 3747-3754.
4. Poller, W., et al. 1992. Cloning of the human α-2-Macroglobulin gene and detection of mutations in two functional domains: the bait region and the thiolester site. Hum. Genet. 88: 313-319.
5. Webb, D.J., et al. 1998. Localization of the binding site for transforming growth factor-β in human α-2-Macroglobulin to a 20-kDa peptide that also contains the bait region. J. Biol. Chem. 273: 13339-13346.
6. Blacker, D., et al. 1998. α-2-Macroglobulin is genetically associated with Alzheimer disease. Nat. Genet. 19: 357-360.

CHROMOSOMAL LOCATION

Genetic locus: A2M (human) mapping to 12p13.31.

PRODUCT

α-2M 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 α-2M shRNA Plasmid (h): sc-40297-SH and α-2M shRNA (h) Lentiviral Particles: sc-40297-V as alternate gene silencing products.

For independent verification of α-2M (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-40297A, sc-40297B and sc-40297C.

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

α-2M siRNA (h) is recommended for the inhibition of α-2M 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

α-2M (H-8): sc-390544 is recommended as a control antibody for monitoring of α-2M 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 α-2M gene expression knockdown using RT-PCR Primer: α-2M (h)-PR: sc-40297-PR (20 µl, 503 bp). 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.