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ADAM10 siRNA (r): sc-270165

BACKGROUND

ADAM (a disintegrin and metalloprotease) proteins are a family of over 30 membrane-anchored, glycosylated, Zn²⁺-dependent proteases that are involved in cell-cell, cell-matrix interface related processes including fertilization, muscle fusion, secretion of TNF α (tumor necrosis factor α), and modulation of the neurogenic function of Notch and Delta. ADAM proteins possess a signal-domain, a pro-domain, a metalloprotease domain, a disintegrin domain (integrin ligand) a cysteine-rich region, an epidermal growth factor-like domain, a transmembrane domain and a cytoplasmic tail. ADAMs are expressed in brain, testis, epididymis, ovary, breast, placenta, liver, heart, lung, bone and muscle, and catalyze proteolysis, adhesion, fusion and intracellular signaling. ADAM10 is a TNF-processing enzyme that cleaves pro-TNF, a membrane-bound precursor protein, at Ala 76-Val 77, which causes membrane shedding of soluble TNF.

REFERENCES

1. Wolfsberg, T.G., et al. 1995. ADAM, a novel family of membrane proteins containing a disintegrin and metalloprotease domain: multipotential functions in cell-cell and cell-matrix interactions. *J. Cell Biol.* 131: 275-278.
2. Rosendahl, M.S., et al. 1997. Identification and characterization of a pro-tumor necrosis factor- α -processing enzyme from the ADAM family of zinc metalloproteases. *J. Biol. Chem.* 272: 24588-24593.
3. Stone, A.L., et al. 1999. Structure-function analysis of the ADAM family of disintegrin-like and metalloproteinase-containing proteins (review). *J. Protein Chem.* 18: 447-465.
4. Primakoff, P. and Myles, D.G. 2000. The ADAM gene family: surface proteins with adhesion and protease activity. *Trends Genet.* 16: 83-87.
5. Online Mendelian Inheritance in Man, OMIM[™]. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 602192. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: Adam10 (rat) mapping to 8q24.

PRODUCT

ADAM10 siRNA (r) 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 ADAM10 shRNA Plasmid (r): sc-270165-SH and ADAM10 shRNA (r) Lentiviral Particles: sc-270165-V as alternate gene silencing products.

For independent verification of ADAM10 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270165A, sc-270165B and sc-270165C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

ADAM10 siRNA (r) is recommended for the inhibition of ADAM10 expression in rat 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

ADAM10 (B-3): sc-28358 is recommended as a control antibody for monitoring of ADAM10 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 ADAM10 gene expression knockdown using RT-PCR Primer: ADAM10 (r)-PR: sc-270165-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.