

PAI-1 siRNA (r): sc-60075

BACKGROUND

PAI-1 and PAI-2 (for plasminogen activator inhibitor-1 and -2) are members of the serpin serine proteinase inhibitor family. PAI-1 and PAI-2 have been shown to regulate uPA (urokinase-type plasminogen activator) and tPA (tissue plasminogen activator), resulting in the inhibition of proteolytic activity. Members of the serpin family generally complex with their target proteinases, then disassociate slowly into cleaved species that fold into stable inactive forms. PAI-1 can fold into the inactive state without cleavage, resulting in the latent form of PAI-1. Activity can be restored to the latent form of PAI-1 through denaturation and renaturation. PAI-2 occurs in secreted and cytosolic forms through facultative polypeptide translocation. uPA is a serine proteinase that is a member of the Trypsin family. It is responsible for the cleavage of plasminogen at the Arg-Val bond to produce plasmin. uPA consists of two chains designated A and B. The A chain can be cleaved, resulting in low and high molecular mass forms of uPA.

REFERENCES

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2. Belin, D., et al. 1989. Facultative polypeptide translocation allows a single mRNA to encode the secreted and cytosolic forms of plasminogen activator inhibitor-2. *EMBO J.* 8: 3287-3294.
3. Schmitt, M., et al. 1991. Human tumor cell urokinase-type plasminogen activator (uPA): degradation of the proenzyme form (pro-uPA) by granulocyte elastase prevents subsequent activation by plasmin. *Adv. Exp. Med. Biol.* 297: 111-128.
4. Mottonen, J., et al. 1992. Structural basis of latency in plasminogen activator inhibitor-1. *Nature* 355: 270-273.
5. Niedbala, M.J. 1993. Cytokine regulation of endothelial cell extracellular proteolysis. *Agents Actions Suppl.* 42: 179-193.
6. Schaefer, B.M., et al. 1995. Differential expression of urokinase-type plasminogen activator (uPA), its receptor (uPA-R), and inhibitor type-2 (PAI-2) during differentiation of keratinocytes in an organotypic coculture system. *Exp. Cell Res.* 220: 415-423.

CHROMOSOMAL LOCATION

Genetic locus: Serpine1 (rat) mapping to 12q12.

PRODUCT

PAI-1 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 PAI-1 shRNA Plasmid (r): sc-60075-SH and PAI-1 shRNA (r) Lentiviral Particles: sc-60075-V as alternate gene silencing products.

For independent verification of PAI-1 (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-60075A, sc-60075B and sc-60075C.

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

PAI-1 siRNA (r) is recommended for the inhibition of PAI-1 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

PAI-1 (C-9): sc-5297 is recommended as a control antibody for monitoring of PAI-1 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 PAI-1 gene expression knockdown using RT-PCR Primer: PAI-1 (r)-PR: sc-60075-PR (20 μ l, 566 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.