

PDIR siRNA (m): sc-62768

BACKGROUND

Oxidoreductase-protein disulfide isomerase (PDI) is a homodimer that catalyzes thiol-disulfide exchange, mediates folding of newly synthesized proteins and functions as a molecular chaperone. PDIR (Protein disulfide isomerase-related protein), also known as PDIA5 (Protein disulfide-isomerase A5), is a 519 amino acid protein that catalyzes the rearrangement of sulfur-sulfur bonds in various proteins. Localized to the lumen of the endoplasmic reticulum (ER), PDIR has an oxidative refolding activity that is specific for α 1-antitrypsin (AAT) and aids in the formation of disulfide bonds in the ER lumen. PDIR contains one ER retention signal at its C-terminus and three thioredoxin (CXXC) motifs which mediate the substrate-specific isomerase, chaperone and redox activity of PDIR.

REFERENCES

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- Horibe, T., et al. 2004. Different contributions of the three CXXC motifs of human protein-disulfide isomerase-related protein to isomerase activity and oxidative refolding. *J. Biol. Chem.* 279: 4604-4611.
- Horibe, T., et al. 2004. Replacement of domain b of human protein disulfide isomerase-related protein with domain b' of human protein disulfide isomerase dramatically increases its chaperone activity. *FEBS Lett.* 566: 311-315.
- Jessop, C.E., et al. 2004. Oxidative protein folding in the mammalian endoplasmic reticulum. *Biochem. Soc. Trans.* 32: 655-658.
- Maniratanachote, R., et al. 2005. Chaperone proteins involved in troglitazone-induced toxicity in human hepatoma cell lines. *Toxicol. Sci.* 83: 293-302.
- Alanen, H.I., et al. 2006. pH dependence of the peptide thiol-disulfide oxidase activity of six members of the human protein disulfide isomerase family. *Antioxid. Redox Signal.* 8: 283-291.

CHROMOSOMAL LOCATION

Genetic locus: *Pdia5* (mouse) mapping to 16 B3.

PRODUCT

PDIR siRNA (m) 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 PDIR shRNA Plasmid (m): sc-62768-SH and PDIR shRNA (m) Lentiviral Particles: sc-62768-V as alternate gene silencing products.

For independent verification of PDIR (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-62768A, sc-62768B and sc-62768C.

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

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

PDIR (B-9): sc-390862 is recommended as a control antibody for monitoring of PDIR 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 PDIR gene expression knockdown using RT-PCR Primer: PDIR (m)-PR: sc-62768-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.