

CyPA siRNA (h): sc-105263

BACKGROUND

The immunosuppressant cyclosporin A (CsA) forms a trimolecular complex with cyclophilin and calcineurins to inhibit calcineurin phosphatase activity. Cyclophilins are conserved, ubiquitous and abundant cytosolic peptidyl-prolyl *cis-trans* isomerases that accelerate the isomerization of XaaPro peptide bonds and the refolding of proteins. Human cyclophilin A (CyPA), an intracellular protein of 165 amino acids, is the target of the CsA and is encoded by a single unique gene conserved from yeast to humans. CyPA is known for its involvement in T cell differentiation and proliferation and is highly expressed in brain. CyPA is incorporated into the virion of the type 1 human immunodeficiency virus (HIV-1) via a direct interaction with the capsid domain of the viral G_αγ polyprotein and is crucial for efficient viral replication. Cyclophilin B (CyPB) is a member of the cyclophilin family with specific N- and C-terminal extensions. Unlike CyPA, CyPB has a signal sequence leading to its translocation in the endoplasmic reticulum. CyPB is secreted in biological fluids such as blood or milk and binds to a specific receptor present on the human lymphoblastic cell line Jurkat and on human peripheral blood lymphocytes.

REFERENCES

- Hasel, K.W., et al. 1991. An endoplasmic reticulum-specific cyclophilin. *Mol. Cell. Biol.* 11: 3484-3491.
- Arber, S., et al. 1992. S-cyclophilin is retained intracellularly via a unique COOH-terminal sequence and colocalizes with the calcium storage protein calreticulin. *J. Cell Biol.* 116: 113-125.
- Pflugl, G., et al. 1993. X-ray structure of a decameric cyclophilin-cyclosporin crystal complex. *Nature* 361: 91-94.

CHROMOSOMAL LOCATION

Genetic locus: PPIA (human) mapping to 7p13.

PRODUCT

CyPA siRNA (h) is a pool of 2 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 CyPA shRNA Plasmid (h): sc-105263-SH and CyPA shRNA (h) Lentiviral Particles: sc-105263-V as alternate gene silencing products.

For independent verification of CyPA (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-105263A and sc-105263B.

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

CyPA siRNA (h) is recommended for the inhibition of CyPA 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 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

CyPA (6-YD13): sc-134310 is recommended as a control antibody for monitoring of CyPA 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 CyPA gene expression knockdown using RT-PCR Primer: CyPA (h)-PR: sc-105263-PR (20 μ l, 550 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Dharan, A., et al. 2016. KIF5B and Nup358 cooperatively mediate the nuclear import of HIV-1 during infection. *PLoS Pathog.* 12: e1005700.
- Sengupta, D., et al. 2017. Modulation of adenylate cyclase signaling in association with MKK3/6 stabilization under combination of SAC and berberine to reduce HepG2 cell survivability. *Apoptosis* 22: 1362-1379.

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.