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SANTA CRUZ BIOTECHNOLOGY, INC.

PNP siRNA (h): sc-45991

BACKGROUND

Purine nucleoside phosphorylase (PNP), also designated inosine phosphorylase, forms a homotrimer. It belongs to the PNP/MTAP phosphorylase family of proteins. Human PNP catalyzes the reversible phosphorolysis of ribonucleosides and 2'-deoxyribonucleosides with specificity for guanine, hypoxanthine, and their analogs. PNP deficiency is a rare autosomal recessive genetic disease associated with a severe defect in T-lymphocyte function and neurologic disorder in children, comprising four percent of combined immunodeficiency cases. Children with PNP deficiency are highly prone to infections, autoimmune disorders, neurological impairment, and cancer.

REFERENCES

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3. Carlucci, F., Tabucchi, A., Aiuti, A., Rosi, F., Floccari, F., Pagani, R. and Marinello, E. 2003. Capillary electrophoresis in diagnosis and monitoring of adenosine deaminase deficiency. Clin. Chem. 49: 1830-1838.
4. Zang, Y., Wang, W.H., Wu, S.W., Ealick, S.E. and Wang, C.C. 2005. Identification of a subversive substrate of trichomonas vaginalis purine nucleoside phosphorylase and the crystal structure of the enzyme-substrate complex. J. Biol. Chem. 280: 22318-22325.
5. Canduri, F., Fadel, V., Dias, M.V., Basso, L.A., Palma, M.S., Santos, D.S. and de Azevedo, W.F., Jr. 2005. Crystal structure of human PNP complexed with hypoxanthine and sulfate ion. Biochem. Biophys. Res. Commun. 326: 335-338.
6. Canduri, F., Fadel, V., Basso, L.A., Palma, M.S., Santos, D.S. and de Azevedo, W.F., Jr. 2005. New catalytic mechanism for human purine nucleoside phosphorylase. Biochem. Biophys. Res. Commun. 327: 646-649.

CHROMOSOMAL LOCATION

Genetic locus: PNP (human) mapping to 14q11.2.

PRODUCT

PNP 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 PNP shRNA Plasmid (h): sc-45991-SH and PNP shRNA (h) Lentiviral Particles: sc-45991-V as alternate gene silencing products.

For independent verification of PNP (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-45991A, sc-45991B and sc-45991C.

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

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

PNP (F-10): sc-365551 is recommended as a control antibody for monitoring of PNP 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 PNP gene expression knockdown using RT-PCR Primer: PNP (h)-PR: sc-45991-PR (20 µl). 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.