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SANTA CRUZ BIOTECHNOLOGY, INC.

POH1 siRNA (m): sc-152368

BACKGROUND

POH1, the human homolog of yeast Pad1, is part of the 26S proteasome which degrades protein targeted for destruction by the ubiquitin pathway. Specifically, POH1 is part of the 19S regulatory cap of the 26S proteasome where it deubiquitinates proteins and allows proteins to pass through the narrow, cylindrical 26S proteasome core. POH1 is most abundantly expressed in heart and skeletal muscle. Transient overexpression of POH1 in mammalian cells confers P-glycoprotein-indedependent resistance to Taxol, doxorubicin, 7-hydroxystaurosporine and ultraviolet light. The gene encoding human POH1 maps to chromosome 2q24.2. The Pad1 homolog in marine sponges (POHL) is upregulated in response to toxins Staurosporin and Taxol. In *Schistosoma mansoni,* the Pad1 homolog SmPOH appears to stabilize c-Jun and elevates the levels of c-Jun for transactivation of AP-1-responsive genes.

REFERENCES

1. Spataro, V., Toda, T., Craig, R., Seeger, M., Dubiel, W., Harris, A.L. and Norbury, C. 1997. Resistance to diverse drugs and ultraviolet light conferred by overexpression of a novel human 26 S Proteasome subunit. J. Biol. Chem. 272:30470-30475.
2. Krasko, A., Kurelec, B., Batel, R., Muller, I.M. and Muller, W.E. 2001. Potential multidrug resistance gene POHL: an ecologically relevant indicator in marine sponges. Environ. Toxicol. Chem. 20:198-204.
3. Nabhan, J.F., Hamdan, F.F. and Ribeiro, P. 2001. A *Schistosoma mansoni* Pad1 homologue stabilizes c-Jun. Mol. Biochem. Parasitol. 116:209-218.
4. Yao, T. and Cohen, R.E. 2002. A cryptic protease couples deubiquitination and degradation by the proteasome. Nature 419:403-407.
5. LocusLink Report (Locus ID: 10213). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: Psmd14 (mouse) mapping to 2 C1.3.

PRODUCT

POH1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see POH1 shRNA Plasmid (m): sc-152368-SH and POH1 shRNA (m) Lentiviral Particles: sc-152368-V as alternate gene silencing products.

For independent verification of POH1 (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-152368A, sc-152368B and sc-152368C.

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.

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

POH1 siRNA (m) is recommended for the inhibition of POH1 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 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

POH1 (SQ-17): sc-100464 is recommended as a control antibody for monitoring of POH1 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 POH1 gene expression knockdown using RT-PCR Primer: POH1 (m)-PR: sc-152368-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.