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PYPAF7 siRNA (m): sc-150004

BACKGROUND

Pyrin-containing Apaf1-like proteins (PYPAFs) are members of the nucleotide-binding site/leucine-rich repeat (NBS/LRR) family of signal transduction proteins that function in apoptotic and inflammatory signaling pathways. PYPAF7, also known as Monarch-1, regulates activation of caspase-1 via ASC protein and promotes activation of NFκB via IKK. PYPAF7 enhances nonclassical and classical MHC class I expression at the level of the promoter, RNA, and protein expression. PYPAF7 is present in the cytoplasm of peripheral blood leukocytes, predominantly eosinophils and granulocytes, with lower levels in monocytes. The PYPAF7 gene maps to human chromosome 19q13.4.

REFERENCES

1. Wang, L., et al. 2002. PYPAF7, a novel PYRIN-containing Apaf1-like protein that regulates activation of NFκB and caspase-1-dependent cytokine processing. *J. Biol. Chem.* 277: 29874-29880.
2. Grenier, J.M., et al. 2002. Functional screening of five PYPAF family members identifies PYPAF5 as a novel regulator of NFκB and caspase-1. *FEBS Lett.* 530: 73-78.
3. Williams, K., et al. 2003. Cutting edge: Monarch-1: a pyrin/nucleotide-binding domain/leucine-rich repeat protein that controls classical and nonclassical MHC class I genes. *J. Immunol.* 170: 5354-5358.
4. Hasegawa, M., et al. 2005. ASC-mediated NFκB activation leading to IL-8 production requires caspase-8 and is inhibited by CLARP. *J. Biol. Chem.* 280: 15122-15130.
5. SWISS-PROT/TrEMBL (P59046). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: Nlrp12 (mouse) mapping to 7 A1.

PRODUCT

PYPAF7 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 PYPAF7 shRNA Plasmid (m): sc-150004-SH and PYPAF7 shRNA (m) Lentiviral Particles: sc-150004-V as alternate gene silencing products.

For independent verification of PYPAF7 (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-150004A, sc-150004B and sc-150004C.

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

PYPAF7 siRNA (m) is recommended for the inhibition of PYPAF7 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

PYPAF7 (A-3): sc-390666 is recommended as a control antibody for monitoring of PYPAF7 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 PYPAF7 gene expression knockdown using RT-PCR Primer: PYPAF7 (m)-PR: sc-150004-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.