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Cryopyrin shRNA (m) Lentiviral Particles: sc-45470-V

BACKGROUND

Cryopyrin interacts selectively with apoptosis-associated speck-like protein containing a CARD domain (ASC). This complex may function as an upstream activator of NF κ B signaling and caspase-1 activation. The complex also inhibits TNF α -induced activation and nuclear translocation of RelA/NF κ B p65. Mutations in Cryopyrin and Pypin proteins are responsible for several autoinflammatory disorders in humans, including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurologic cutaneous and articular syndrome (CINCA).

REFERENCES

1. Dode, C. 2002. New mutations of CIAS1 that are responsible for Muckle-Wells syndrome and familial cold urticaria: a novel mutation underlies both syndromes. *Am. J. Hum. Genet.* 70: 1498-1506.
2. Feldmann, J. 2002. Chronic infantile neurological cutaneous and articular syndrome is caused by mutations in CIAS1, a gene highly expressed in polyphosphonuclear cells and chondrocytes. *Am. J. Hum. Genet.* 71: 198-203.
3. Rosengren, S. 2005. Expression and regulation of Cryopyrin and related proteins in rheumatoid arthritis synovium. *Ann. Rheum. Dis.* 64: 708-714.
4. Bihl, T. 2005. The T348M mutated form of cryopyrin is associated with defective lipopolysaccharide-induced interleukin 10 production in CINCA syndrome. *Ann. Rheum. Dis.* 64: 1380-1381.
5. Yu, J.W. 2005. Cryopyrin and Pypin activate caspase-1, but not NF κ B, via ASC oligomerization. *Cell Death Differ.* 13: 236-249.

CHROMOSOMAL LOCATION

Genetic locus: *Cias1* (mouse) mapping to 11 B1.3.

PRODUCT

Cryopyrin shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Cryopyrin siRNA (m): sc-45470 and Cryopyrin shRNA Plasmid (m): sc-45470-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

Cryopyrin shRNA (m) Lentiviral Particles is recommended for the inhibition of Cryopyrin expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Cryopyrin (H-66): sc-66846 is recommended as a control antibody for monitoring of Cryopyrin 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Cryopyrin gene expression knockdown using RT-PCR Primer: Cryopyrin (m)-PR: sc-45470-PR (20 μ l, 464 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.