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ISG15 shRNA (h) Lentiviral Particles: sc-43869-V

BACKGROUND

Interferon-induced 15 kDa protein (ISG15) acts as ubiquitin by conjugating to intracellular target proteins such as JAK1 or MAPK3/ERK1 through an enzyme pathway distinct from that of ubiquitin. ISG15 shows specific chemotactic activity towards neutrophils and activates them to induce the release of eosinophil chemotactic factors. ISG15 is also involved in paracrine, autocrine and endocrine mechanisms by inducing IFN- γ secretion by monocytes and macrophages, as in cell-to-cell signaling. ISG15 is a cytoplasmic protein expressed mainly in muscle, epithelial, neuronal and lymphoid cells.

REFERENCES

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3. Kim, K.I., et al. 2003. ISG15, not just another ubiquitin-like protein. *Biochem. Biophys. Res. Commun.* 307: 431-434.
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5. Austin, K.J., et al. 2004. Localization of ISG15 and conjugated proteins in bovine endometrium using immunohistochemistry and electron microscopy. *Endocrinology* 145: 967-975.
6. Ritchie, K.J., et al. 2004. Role of ISG15 protease UBP43 (USP18) in innate immunity to viral infection. *Nat. Med.* 10: 1374-1378.
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CHROMOSOMAL LOCATION

Genetic locus: ISG15 (human) mapping to 1p36.33.

PRODUCT

ISG15 shRNA (h) 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 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ISG15 siRNA (h): sc-43869 and ISG15 shRNA Plasmid (h): sc-43869-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

ISG15 shRNA (h) Lentiviral Particles is recommended for the inhibition of ISG15 expression in human 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

ISG15 (A-4): sc-514964 is recommended as a control antibody for monitoring of ISG15 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ISG15 gene expression knockdown using RT-PCR Primer: ISG15 (h)-PR: sc-43869-PR (20 μ l, 452 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.