



ISG15 siRNA (m): sc-60049

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, as in cell-to-cell signaling, possibly partly by inducing IFN- γ secretion by monocytes and macrophages. ISG15 is a cytoplasmic protein expressed mainly in muscle, epithelia, neurons and lymphoid cells.

REFERENCES

1. Knight, E., Jr., et al. 1991. IFN-induced 15 kDa protein is released from human lymphocytes and monocytes. *J. Immunol.* 146: 2280-2284.
2. Loeb, K.R., et al. 1992. The interferon-inducible 15 kDa ubiquitin homolog conjugates to intracellular proteins. *J. Biol. Chem.* 267: 7806-7813.
3. Kim, K.I., et al. 2003. ISG15, not just another ubiquitin-like protein. *Biochem. Biophys. Res. Commun.* 307: 431-434.
4. Liu, M., et al. 2003. Proteasomes modulate conjugation to the ubiquitin-like protein, ISG15. *J. Biol. Chem.* 278: 1594-1602.
5. Ritchie, K.J., et al. 2004. Role of ISG15 protease UBP43 (USP18) in innate immunity to viral infection. *Nat. Med.* 10: 1374-1378.
6. Austin, K.J., et al. 2004. Localization of ISG15 and conjugated proteins in bovine endometrium using immunohistochemistry and electron microscopy. *Endocrine* 145: 967-975.

CHROMOSOMAL LOCATION

Genetic locus: *Isg15* (mouse) mapping to 4 E2.

PRODUCT

ISG15 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ISG15 shRNA Plasmid (m): sc-60049-SH and ISG15 shRNA (m) Lentiviral Particles: sc-60049-V as alternate gene silencing products.

For independent verification of ISG15 (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-60049A, sc-60049B and sc-60049C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ISG15 siRNA (m) is recommended for the inhibition of ISG15 expression in mouse cells.

GENE EXPRESSION MONITORING

ISG15 (F-9): sc-166755 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 reagents are recommended:

- 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ 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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).
- 3) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.
- 4) Immunohistochemistry: use m-IgG κ BP-HRP: sc-516102 with DAB, 50X: sc-24982 and Immunohistomount: sc-45086, or Organo/Limonene Mount: sc-45087.

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 μ M in 66 μ l. Each contains 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ISG15 gene expression knockdown using RT-PCR Primer: ISG15 (m)-PR: sc-60049-PR (20 μ l). 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 or our catalog for detailed protocols and support products.