#12621 Store at -20°C

SignalSilence® ISG15 siRNA II

☑ 10 µM in 300 µl (3 nmol)

Cell Signaling TECHNOLOGY®

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New 05/13

For Research Use Only. Not For Use In Diagnostic Procedures.

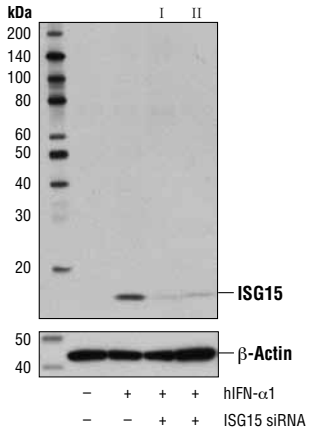
Species Cross-Reactivity: H

Description: SignalSilence® ISG15 siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit ISG15 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

Background: Interferon-stimulated 15 kDa protein (ISG15), also known as ubiquitin cross-reactive protein (UCRP), is a member of the ubiquitin-like protein family and functions in various biological pathways from pregnancy to innate immune responses (1). Expression of ISG15 is stimulated by cellular exposure to type 1 interferons α and β, in addition to infection with viruses such as influenza B (2,3). After exposure to type I interferons, both lymphocytes and monocytes, in addition to some fibroblasts and epithelial cells, release ISG15 into culture medium (1,4). ISG15 has been shown to function as a cytokine, stimulating interferon γ secretion by monocytes and macrophages, proliferation of natural killer cells, and chemotactic responses in neutrophils (4,5). ISG15 has also been shown to function intracellularly, being covalently conjugated to other proteins by E1 (Ube1L), E2 (UbcH8) and E3 ligases via a multi-step process analogous to ubiquitination (6,7). ISG15 is removed from proteins by the ubiquitin processing protease Ubp43 (8). ISG15-protein conjugation (ISGylation) is induced by type 1 interferons, and target proteins include the serine protease inhibitor Serpin 2A, PLCγ1, ERK1/2, Jak1 and Stat1 (9,10). Unlike ubiquitination, ISGylation does not target proteins for degradation, rather ISGylation increases Jak1 and Stat1 activity, enhancing the cellular response to interferons (11).

Directions for Use: CST recommends transfection with 100 nM SignalSilence® ISG15 siRNA II 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300 µl per well.



Western blot analysis of lysates from A549 cells, untreated (-), transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® ISG15 siRNA I #12557 (+), or SignalSilence® ISG15 siRNA II (+), followed by treatment with Human Interferon-α1 (hIFN-α1) #8927 (40 ng/ml, 24 hr; +), using ISG15 (22D2) Rabbit mAb #2758 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). The ISG15 (22D2) Rabbit mAb confirms silencing of ISG15 expression, while the β-Actin (D6A8) Rabbit mAb is used as a loading control.

Quality Control: Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

Entrez-Gene ID #9636
Swiss-Prot Acc. #P05161

Storage: ISG15 siRNA II is supplied in RNAse-free water. *Aliquot and store at -20°C.*

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Ritchie, K.J. and Zhang, D.E. (2004) *Semin. Cell Dev. Biol.* 15, 237-246.

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(4) Knight, E. and Cordova, B. (1991) *J. Immunol.* 146, 2280-2284.

(5) D'Cunha, J. et al. (1996) *Proc. Natl. Acad. Sci. USA* 93, 211-215.

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(11) Malakhova, O.A. et al. (2003) *Genes Dev.* 17, 455-460.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.