#2743 Store at –20°C

ISG15 Antibody

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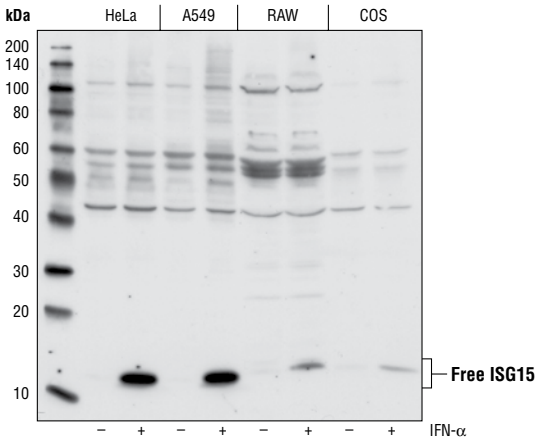
Entrez-Gene ID #9636
Swiss-Prot Acc. #P05161

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, F, E-P Endogenous	H, M, Mk	15 kDa	Rabbit**

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).

Specificity/Sensitivity: This antibody detects endogenous levels of both free and conjugated ISG15 protein. The antibody does not cross-react with other ubiquitin family members, including ubiquitin, SUMO1, SUMO2, SUMO3 and NEDD8.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino acids from human ISG15 protein. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of lysates from HeLa, A549, RAW and COS cells, treated with or without IFN-α (1000 U/mL) for 24 hours, using ISG15 antibody.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Flow Cytometry	1:200
ELISA-Peptide	1:100

For application specific protocols please see the web page for this product at www.cellsignal.com.

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.

(2) Korant, B.D. et al. (1984) *J. Biol. Chem.* 259, 14835-14839.

(3) Haas, A.L. et al. (1987) *J. Biol. Chem.* 262, 11315-11323.

(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.

(6) Loeb, K.R. and Haas, A.L. (1992) *J. Biol. Chem.* 267, 7806-7813.

(7) Zhao, C. et al. (2005) *Proc. Natl. Acad. Sci. USA* 102, 10200-10205.

(8) Malakhov, M.P. et al. (2002) *J. Biol. Chem.* 277, 9976-9981.

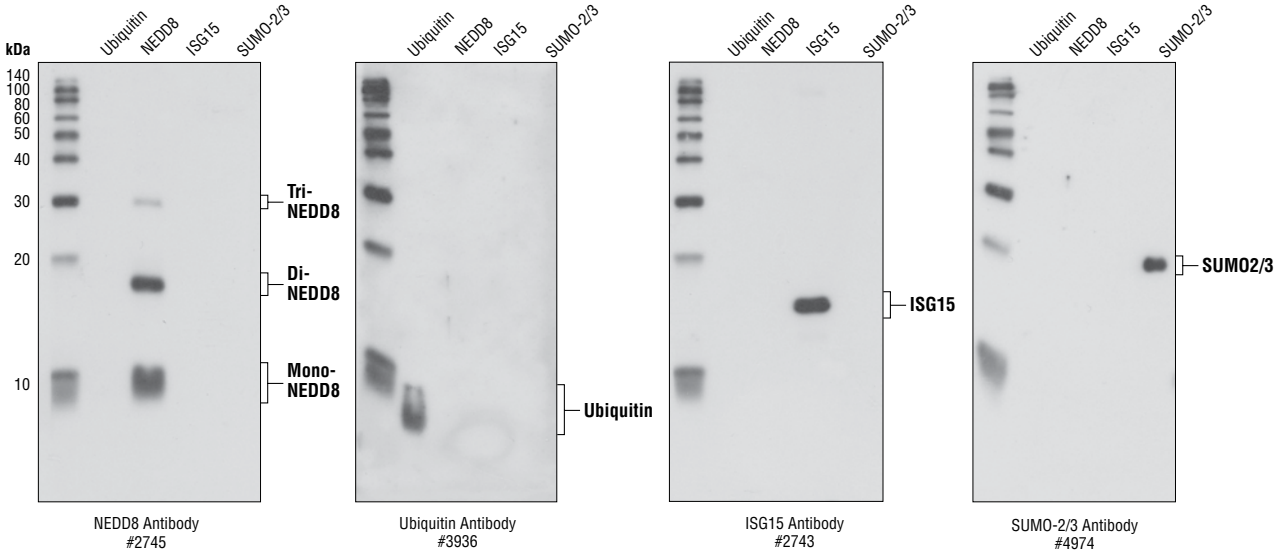
(9) Malakhov, M.P. et al. (2003) *J. Biol. Chem.* 278, 16608-16613.

(10) Hamerman, J.A. et al. (2002) *J. Immunol.* 168, 2415-2423.

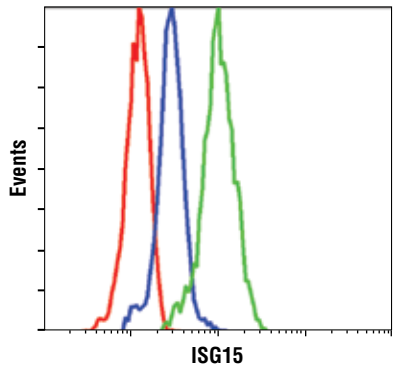
(11) Malakhova, O.A. et al. (2003) *Genes Dev.* 17, 455-460.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

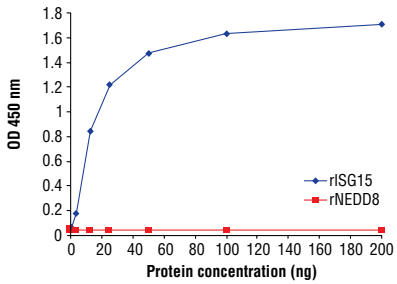
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Western blot analysis of NEDD8, Ubiquitin, ISG15 and SUMO-2/3 recombinant proteins (5 ng each), using NEDD8 (#2745) , Ubiquitin (#3936), ISG15 (#2743) and SUMO-2/3 (#4974) Antibodies.



Flow cytometric analysis of Hela cells, untreated (blue) or IFN-α treated (green), using ISG15 antibody compared to a nonspecific negative control antibody (red).



The relationship between recombinant ISG15 protein concentration and assay optical density readings. Recombinant NEDD8 protein was used as a negative control.