

Anti-ISG15 (RABBIT) Antibody ISG15 Antibody Catalog # ASR4394

## **Specification**

# Anti-ISG15 (RABBIT) Antibody - Product Information

Host Conjugate Target Species Reactivity Clonality Application Application Note	Rabbit Unconjugated Human Human Polyclonal WB, IHC, E, I, LCI This purified polyclonal antibody reacts with human ISG15 by western blot and ELISA. Although not tested, this antibody is likely functional in immunohistochemistry and immunoprecipitation. This antibody using the specified conditions may recognize other prominent intrinsic bands (UBLs or conjugates), especially at lower dilutions. An 18.5 kDa band corresponding to human ISG15 is detected. IFNα or IFNß stimulated HeLa cell lysates can be used as a positive				
Physical State Buffer	Lyophilized 0.02 M Potassium Phosphate, 0.15 M				
Builei	Sodium Chloride, pH 7.2				
Immunogen	This purified antibody was prepared from rabbit serum after repeated immunizations with recombinant human ISG15 protein.				
Reconstitution Volume	100 μL				
Reconstitution Buffer	Restore with deionized water (or equivalent)				
Preservative	0.01% (w/v) Sodium Azide				

## Anti-ISG15 (RABBIT) Antibody - Additional Information

Gene ID 9636

Other Names 9636

**Purity** 

This product is an IgG fraction antibody purified from monospecific antiserum by a multi-step process which includes delipidation, salt fractionation and ion exchange chromatography followed by extensive dialysis against the buffer stated above. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum.

**Storage Condition** 



Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

#### **Precautions Note**

This product is for research use only and is not intended for therapeutic or diagnostic applications.

## Anti-ISG15 (RABBIT) Antibody - Protein Information

Name ISG15 (HGNC:4053)

#### Synonyms G1P2, UCRP

#### Function

Ubiquitin-like protein which plays a key role in the innate immune response to viral infection either via its conjugation to a target protein (ISGylation) or via its action as a free or unconjugated protein (PubMed:<a href="http://www.uniprot.org/citations/27564865"">http://www.uniprot.org/citations/27564865</a>

target="\_blank">27564865</a>, PubMed:<a href="http://www.uniprot.org/citations/39465252" target="\_blank">39465252</a>). ISGylation involves a cascade of enzymatic reactions involving E1, E2, and E3 enzymes which catalyze the conjugation of ISG15 to a lysine residue in the target protein (PubMed:<a href="http://www.uniprot.org/citations/33727702"

target="\_blank">33727702</a>). Its target proteins include IFIT1, MX1/MxA, PPM1B, UBE2L6, UBA7, CHMP5, CHMP2A, CHMP4B and CHMP6. Isgylation of the viral sensor IFIH1/MDA5 promotes IFIH1/MDA5 oligomerization and triggers activation of innate immunity against a range of viruses, including coronaviruses, flaviviruses and picornaviruses (PubMed:<a

href="http://www.uniprot.org/citations/33727702" target="\_blank">33727702</a>). Can also isgylate: EIF2AK2/PKR which results in its activation, RIGI which inhibits its function in antiviral signaling response, EIF4E2 which enhances its cap structure-binding activity and translation-inhibition activity, UBE2N and UBE2E1 which negatively regulates their activity, IRF3 which inhibits its ubiquitination and degradation and FLNB which prevents its ability to interact with the upstream activators of the INK cascade thereby inhibiting IFNA-induced INK signaling. Exhibits antiviral activity towards both DNA and RNA viruses, including influenza A, HIV-1 and Ebola virus. Restricts HIV-1 and ebola virus via disruption of viral budding. Inhibits the ubiquitination of HIV-1 Gag and host TSG101 and disrupts their interaction, thereby preventing assembly and release of virions from infected cells. Inhibits Ebola virus budding mediated by the VP40 protein by disrupting ubiquitin ligase activity of NEDD4 and its ability to ubiquitinate VP40. ISGylates influenza A virus NS1 protein which causes a loss of function of the protein and the inhibition of virus replication. The secreted form of ISG15 can: induce natural killer cell proliferation, act as a chemotactic factor for neutrophils and act as a IFN-gamma-inducing cytokine playing an essential role in antimycobacterial immunity. The secreted form acts through the integrin ITGAL/ITGB2 receptor to initiate activation of SRC family tyrosine kinases including LYN, HCK and FGR which leads to secretion of IFNG and IL10; the interaction is mediated by ITGAL (PubMed:<a href="http://www.uniprot.org/citations/29100055" target=" blank">29100055</a>).

#### **Cellular Location**

Cytoplasm. Secreted Note=Exists in three distinct states: free within the cell, released into the extracellular space, or conjugated to target proteins

#### **Tissue Location**

Detected in lymphoid cells, striated and smooth muscle, several epithelia and neurons. Expressed in neutrophils, monocytes and lymphocytes. Enhanced expression seen in pancreatic adenocarcinoma, endometrial cancer, and bladder cancer, as compared to non-cancerous tissue. In bladder cancer, the increase in expression exhibits a striking positive correlation with more advanced stages of the disease.



# Anti-ISG15 (RABBIT) Antibody - Protocols

Provided below are standard protocols that you may find useful for product applications.

- <u>Western Blot</u>
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

# Anti-ISG15 (RABBIT) Antibody - Images

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Western blot validation of host protein regulation. A, HeLa cells were mock-infected or infected for 24 h, or B, for indicated periods of time, harvested and lysed with 0.5% NP-40 detergent. The cytosolic and nuclear fractions were separately purified, dissolved in SDS electrophoresis sample buffer, and proteins resolved in 10% (A), or in 4-16% gradient (B) SDS-PAGE, transferred to PVDF, and probed with indicated antibodies. Antibody binding was detected with HRP-conjugated secondary antibodies and ECL, and visualized with an Alpha Innotech FluorChemQ MultiImage III instrument. Molecular weight standards are indicated at left and SILAC-measured ratios are indicated on right in A. \*: not detected in indicated fraction; †: based on single peptide only. Figure provided by CiteAb. Source: Virol J, PMID: 23799967.

## Anti-ISG15 (RABBIT) Antibody - Background

Ubiquitin-like proteins fall into two classes: the first class, ubiquitin-like modifiers (UBLs) function as modifiers in a manner analogous to that of ubiquitin. Examples of UBLs are SUMO, Rub1 (also called Nedd8), Apg8 and Apg12. Proteins of the second class include parkin, RAD23 and DSK2, are designated ubiquitin-domain proteins (UDPs). These proteins contain domains that are related to ubiquitin but are otherwise unrelated to each other. In contrast to UBLs, UDPs are not conjugated to other proteins. ISG15 (Interferon Stimulating Gene-15) shows no amino acid sequence homology to cytokines and is synthesized as a precursor that is activated through processing by a thiol protease. ISG15 is secreted by monocytes and lymphocytes. Synthesis is induced in response to IFN-a or IFN-b or IFN-¥, but not IFN-g. ISG15 expression is induced also by overexpression of some interferon regulatory factors that have been shown to play a role in the transcriptional regulation of



IFN genes. ISG15 is secreted also by cell lines of monocyte (U937 cell line), T-lymphocyte, B-lymphocyte (DAUDI cells), human fibroblasts, and epithelial origins. The induction of terminal differentiation in human melanoma cells is associated, among other things, with alterations in the expression of ISG15. Intracellularly ISG15 has been shown to function as a ubiquitin homologue. It is known also as UCRP (ubiquitin cross-reactive protein). Serpin 2a (spi2a), a member of the serine protease inhibitor (serpin) protein family that is highly induced in macrophages during bacillus Calmette-Guerin infection has been shown to bind ISG15. ISG15 has been shown to modulate immune cell function. It possesses activities of cytokines and induces production of IFN-g. It enhances proliferation and functions of natural killer and LAK cells.