

## Product Specification Sheet

**Product:** IgG fraction of Anti-ISG15 (Human) (Rabbit)

**Code:** 200-401-438 **Lot #:** 13033

**Size:** 500 g

**Antibody Concentration:** 5.0 mg/ml (by UV absorbance at 280 nm)

**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

**Stabilizer:** None **Preservative:** 0.01% (w/v) Sodium Azide

**Storage Conditions:** Store vial at 4° C prior to restoration. Restore with 0.1 ml of deionized water (or equivalent). 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. Expiration date is one (1) year from date of restoration.

**Background Information:** 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, AD23 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- $\alpha$  or IFN- $\beta$  or IFN- $\gamma$ , but not IFN- $\delta$ . 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- $\gamma$ . It enhances proliferation and functions of natural killer and LAK cells.

**Application Note(s):** 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). Other intrinsic bands are readily detectable at lower dilutions.

**Recommended Dilution(s):** For immunoblotting a 1:200 dilution is recommended. An 18.5 kDa band corresponding to human ISG15 is detected. Most human cell lysates can be used as a positive control without induction or stimulation. For ELISA a 1:2,000 to 1:10,000 dilution is recommended. Researchers should determine optimal titers for other applications.

**Purity and Specificity:** 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.

**Related Link(s):**

UBL [protein-protein interactions](http://depts.washington.edu/sfields/yplm/data/Nature.html) in *S.cerevisiae*. (<http://depts.washington.edu/sfields/yplm/data/Nature.html>)  
ISG15 [primary sequence](http://harvester.embl.de/harvester/AAN8/AAN86983.htm) from EMBL as 165 aa, molecular weight: 17914 Da  
(<http://harvester.embl.de/harvester/AAN8/AAN86983.htm>)

**Reference(s):**

D'Cunha, J. et al. (1996) Immunoregulatory properties of ISG15 interferon-induced cytokine. *Proc. Natl. Acad. Sci. USA* **93**; 211-215.

Potter J.L. et al (1999) Precursor Processing of Pro-ISG15/UCRP, an Interferon--induced Ubiquitin-like Protein. *J Biol Chem* **274**;35, 25061-25068.

Malakhov MP et al (2002) UBP43 (USP18) specifically removes ISG15 from conjugated proteins. *J Biol Chem.* **277**(12):9976-81.

Muller, S. , Hoegge, C. , Pyrowolakis, G. and Jentsch, S. (2001) SUMO, ubiquitin's mysterious cousin. *Nat Rev Mol Cell Biol*, 2(3): 202-10.

Liakopoulos D et al. (1998). A novel protein modification pathway related to the ubiquitin system. *EMBO J.* 15;**17**(8):2208-14.

Jentsch S, Pyrowolakis G. (2000) Ubiquitin and its kin: how close are the family ties? *Trends Cell Biol.* **10**(8):335-42.

**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.