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Datasheet for 200-401-438

ISG15 Antibody

Overview

Description:	Anti-ISG15 (RABBIT) Antibody - 200-401-438
Item No.:	200-401-438
Size:	500 µg
Applications:	ELISA, WB, Microarray
Reactivity:	Human
Host Species:	Rabbit

Product Details

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.
Synonyms:	rabbit anti-ISG15 Antibody, G1P2 antibody, IFI 15 antibody, IFI15 antibody, Interferon alpha inducible protein antibody, Interferon induced 15 kDa protein antibody
Host Species:	Rabbit
Clonality:	Polyclonal

Format:	IgG

Target Details

Gene Name:	ISG15
Reactivity:	Human
Immunogen Type:	Recombinant Protein
Immunogen:	This purified antibody was prepared from rabbit serum after repeated immunizations with recombinant human ISG15 protein.
Purity/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.
Relevant Links:	• NCBI - 4826774 • UniProtKB - P05161 • GeneID - 9636

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	Microarray (Based on references)
Application Note:	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 control.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:2,000 - 1:10,000
IHC:	User Optimized
WB:	1:200 - 1:1,000

Formulation

Physical State:	Lyophilized

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
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None
Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

Shipping Condition:	Ambient
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.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

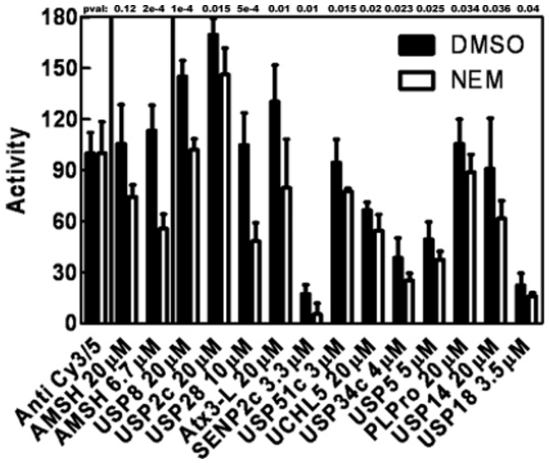
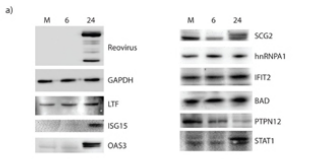


Figure
Thirteen enzymes display deISGylase activity postimmobilization. ISG15-VME arrays identified a larger repertoire of active enzymes: AMSH, USP8c, USP2c, USP28, Atx3-L, SENP2c, USP51, UCHL5, USP34c, USP5, PLPro, USP14, and USP18. ISG15-VME arrays utilized rabbit anti-ISG15 (p/n 200-401-438) 1:49 in PBST. Array signals were quantified, background corrected, normalized to the median anti-Cy3/Cy5 signal, and summarized by their median. Error bars represent the standard deviation of the six signals representing each enzyme/dilution combination.
The p values are from Student's t test where the null hypothesis was [signal DMSO] = [signal NEM]. Fig5.
PMID: 20956615

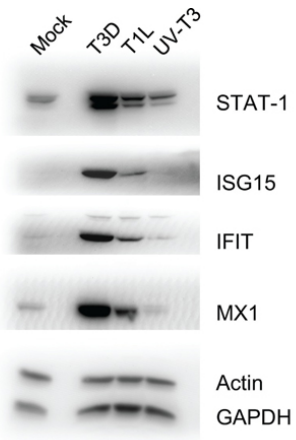


b)

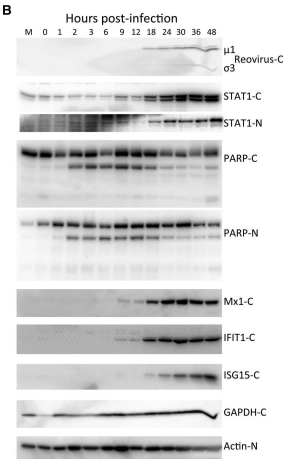
	6hpi SILAC L:H ratio Experiment					24hpi SILAC L:H ratio Experiment				
HGNC	1	2	3	Avg	WB	1	2	3	Avg	WB
GAPDH	1.11	0.92	1.24	1.09	1.02	1.09	1.02	1.16	1.09	0.98
LTF	8.88	ND*	ND	8.88	1.74	22.0	4.20	7.19	11.13	1.89
ISG15	ND	ND	ND	ND	2.45	7.24	4.06	2.66	4.65	16.48
OAS3	ND	ND	ND	ND	0.76	3.96	7.51	ND	5.74	10.59
SCG2	ND	ND	ND	ND	0.51	7.08	8.19	ND	7.64	1.96
hnRNPA1	1.11	0.95	0.94	1.00	1.36	1.21	1.11	0.83	1.05	1.02
IFIT2	ND	ND	ND	ND	0.95	4.22	ND	3.02	3.62	1.28
BAD	2.71	4.52	ND	3.61	0.83	0.76	ND	ND	0.76	0.54
PTPN12	0.40	ND	ND	0.40	0.78	0.29	ND	ND	0.29	0.38
STAT1	1.31	0.94	ND	1.11	1.24	2.16	1.65	4.14	2.38	2.73

*ND: not detected

Western Blot
Validation of SILAC-determined protein abundances.
(a) Western blot analyses of selected proteins. Mock-infected and T1L-infected cells were harvested at 24hpi, lysed with 0.5% NP-40, and 20–80 µg of each cytosolic fraction resolved in each lane of 10×6.5×0.1 cm 10% SDS-mini-PAGE. Proteins were transferred to PVDF membranes, blocked, probed with various indicated primary antibodies, developed with appropriate secondary antibodies, and visualized with an Alpha Innotech FluorChemQ MultiImage III instrument. (b) Densitometry analysis comparison to SILAC L:H average ratios for the ten different host proteins in (a). Most of the Western blot results correlated to the regulation of the proteins observed in SILAC (whether or not they are up or down regulated). All of the proteins tested by Western blot were also identified at 24hpi in SILAC and were therefore used for confirmation. Some of these proteins were also identified at 6hpi in SILAC and these were used for WB comparison. (GAPDH – glyceraldehyde-3-phosphate dehydrogenase, LTF – lactotransferrin, ISG15 – interferon-stimulated protein 15kDa, OAS3 – 2'–5'oligoadenylate synthetase 3, SCG2 – secretogranin II, hnRNPA1 – heterogeneous nuclear ribonucleoprotein A1, IFIT2 – Interferon-induced protein with tetratricopeptide repeats 2, BAD – Bcl-2 binding component 6, PTPN12 – protein tyrosine phosphatase, non-receptor type 12, STAT1 – signal transducer and activator of transcription 1). Fig 4.
PMID: 23240068

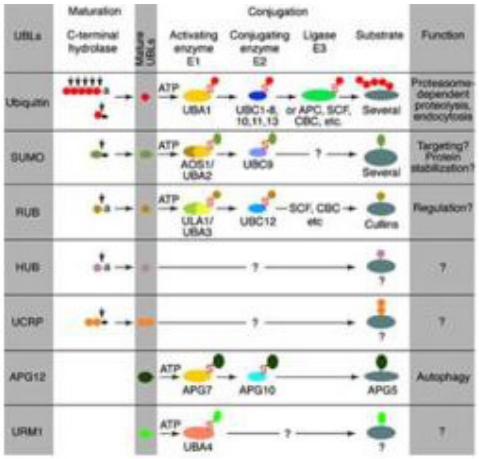


Western Blot
Immunoblot analyses of indicated host proteins in HeLa cells mock-infected, or infected with T3D or T1L, or treated with UV-inactivated T3D. Cells were harvested and lysed with 0.5% NP-40 detergent, nuclei removed, and cytosolic fractions dissolved in SDS electrophoresis sample buffer, resolved in 10% mini SDS-PAGE, transferred to PVDF, and probed with various antibodies. Bands were visualized, and intensities measured, with an Alpha Innotech FluorChem®Q MultiImage® III instrument. Fig 2.
PMID: 25905045



Western Blot

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.



Pathway

Most modifiers mature by proteolytic processing from inactive precursors (a; amino acid). Arrowheads point to the cleavage sites. Ubiquitin is expressed either as polyubiquitin or as a fusion with ribosomal proteins. Conjugation requires activating (E1) and conjugating (E2) enzymes that form thiolesters (S) with the modifiers. Modification of cullins by RUB involves SCF(SKP1/cullin-1/F-box protein) /CBC(cullin-2/elongin B/elonginC) -like E3 enzymes that are also involved in ubiquitination. In contrast to ubiquitin, the UBLs do not seem to form multi-UBL chains. UCRP(ISG15) resembles two ubiquitin moieties linked head-to-tail. Whether HUB1 functions as a modifier is currently unclear. APG12 and URM1 are distinct from the other modifiers because they are unrelated in sequence to ubiquitin. Data contributed by S.Jentsch.

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