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# **Certificate of Analysis**

## Anti-Human ISG15 (MAb)

**Catalog No:** 21900-1 **Lot No:** 7541 **Size:** 100 μg/vial

Description: Mouse Monoclonal Antibody against Human ISG15 Clone: 2.1 Volume: 0.100 ml Concentration: 1 mg/ml Buffer: 50mM Tris HCI, 75mM NaCI, 50% Glycerol pH 8 Antigen: Human ISG15 Isotype: Mouse IgG1 kappa Endotoxin: < 1 EU/µg Purity: > 95% Purification Method: Protein G purified

## Tested Applications: Western blot

**Suggested concentration:** In our hands good visualization of ISGylated proteins was obtained using an antibody concentration of 1  $\mu$ g/ml; however, 0.1  $\mu$ g/ml or lower can be used to give reasonable visualization of the free ISG band. *Optimal dilutions should be determined by each laboratory for each application.* 



**Figure 1. Western Blot.** Control (C) and Treated (T) 40 µg samples from A549, Ovcar and HeLa cell lysates were run alongside 50 ng recombinant ISG15 and 100 µg ubiquitin under reducing conditions in a 12% SDS-PAGE gel. Cells were incubated for 48 hours with or without 1000 U/ml of Human IFN beta 1a (PBL cat# 11410-2). No cross-reactivity was observed with ubiquitin at tested concentrations up to 100 µg. The antibody was additionally tested on Daudi and U937 cell lysates. Please contact us for further information.

#### **References:**

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- 2. C Dieterich and DA Relman. "Modulation of the Host Interferon Response and ISGylation Pathway by B. pertussis Filamentous Hemagglutinin," *PLoS ONE*, 2011, 6(11): e27535.
- 3. YB Zhang, YL Wang, and JF Gui. "Identification and characterization of two homologues of interferon-stimulated gene ISG15 in crucian carp," *Fish and Shellfish Immunology*, 2007, 23(1): 52-61.
- 4. T Takeuchi, T Kobayashi, S Tamura, and H Yokosawa. "Negative regulation of protein phosphatase 2Cβ by ISG15 conjugation," *FEBS Letters*, 2006, 580(18): 4521-4526.
- 5. MP Malakhov, K II Kim, OA Malakhova, BS Jacobs, EC Borden, and D Zhang. "High-throughput Immunoblotting: Ubiquitin-like Protein ISG15 Modifies Key Regulators of Signal Transduction," *JBC*, 2003, 278, 16608-16613.
- 6. J D'Cunha, S Ramanujam, RJ Wagner, PL Witt, E Knight, Jr and EC Borden. "*In vitro* and *in vivo* secretion of human ISG15, an IFN-induced immunomodulatory cytokine," *Journal of Immunology*, 1996, 157:4100.
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#### Shipping Conditions: Wet Ice

### Physical State of Product During Shipping: Liquid

**Storage Conditions/Comments:** After receipt, this product should be kept at -20°C for retention of full activity. Do not store below -20°C. For more information on protein handling, visit our Resource Library at <u>www.pblassaysci.com</u>.

Authorization

Released by:

Date: October 19, 2022

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