# abcam

## Product datasheet

## Anti-ISG15 antibody [EPR3446] ab133346





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Overview

**Product name** Anti-ISG15 antibody [EPR3446]

Rabbit monoclonal [EPR3446] to ISG15 **Description** 

**Host species** Rabbit

**Tested applications** Suitable for: WB, ICC/IF

Unsuitable for: Flow Cyt, IHC-P or IP

Species reactivity Reacts with: Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Daudi whole cell lysate (ab3951), IFN treated wild type HAP1 cell lysate, HeLa (untreated)

and HeLa treated with 10 ng/ml IFN- a1 for 16 hours whole cell lysates ICC/IF: HeLa cells

**General notes** This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

**Properties** 

**Form** 

Storage instructions Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

Storage buffer pH: 7.2

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

**Purity** Tissue culture supernatant

Clonality Monoclonal
Clone number EPR3446
Isotype IqG

#### **Applications**

## The Abpromise guarantee

Our Abpromise guarantee covers the use of ab133346 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000 - 1/10000. Detects a band of approximately 15 kDa (predicted molecular weight: 18 kDa).
ICC/IF		1/100 - 1/250.

**Application notes** 

Is unsuitable for Flow Cyt, IHC-P or IP.

## Target

### **Function**

Ubiquitin-like protein that is conjugated to intracellular target proteins after IFN-alpha or IFN-beta stimulation. Its enzymatic pathway is partially distinct from that of ubiquitin, differing in substrate specificity and interaction with ligating enzymes. ISG15 conjugation pathway uses a dedicated E1 enzyme, but seems to converge with the Ub conjugation pathway at the level of a specific E2 enzyme. Targets include STAT1, SERPINA3G/SPI2A, JAK1, MAPK3/ERK1, PLCG1, EIF2AK2/PKR, MX1/MxA, and RIG-1. Deconjugated by USP18/UBP43. Shows specific chemotactic activity towards neutrophils and activates them to induce release of eosinophil chemotactic factors. May serve as a trans-acting binding factor directing the association of ligated target proteins to intermediate filaments. May also be involved in autocrine, paracrine and endocrine mechanisms, as in cell-to-cell signaling, possibly partly by inducing IFN-gamma secretion by monocytes and macrophages. Seems to display antiviral activity during viral infections.

In response to IFN-tau secreted by the conceptus, may ligate to and regulate proteins involved in the release of prostaglandin F2-alpha (PGF), and thus prevent lysis of the corpus luteum and maintain the pregnancy.

**Tissue specificity** 

Detected in lymphoid cells, striated and smooth muscle, several epithelia and neurons.

Sequence similarities

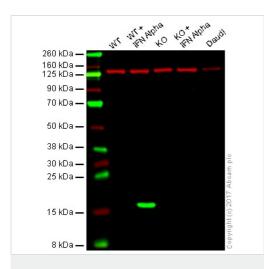
Contains 2 ubiquitin-like domains.

**Cellular localization** 

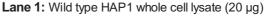
Cytoplasm. Secreted. UCRP conjugates seem to be noncovalently associated with the

intermediate filaments and distributed in a punctate pattern. Also secreted.

#### **Images**



Western blot - Anti-ISG15 antibody [EPR3446] (ab133346)



Lane 2: ISG15 IFN treated wild type HAP1 whole cell lysate (20  $\mu g$ )

Lane 3: ISG15 (KO) whole cell lysate (20 µg)

Lane 4: ISG15 (KO) IFN alpha treated whole cell lysate (20 µg)

Lane 5: Daudi whole cell lysate (20 µg)

**Lanes 1 - 5:** Merged signal (red and green). Green - ab133346 observed at 18 kDa. Red - loading control, <u>ab18058</u>, observed at 130 kDa.

ab133346 was shown to specifically react with ISG15 in IFN alpha1 induced wild-type HAP1cells. Loss of signal was observed when knockout cell line was used. Wild-type and HAP1 IFN treated knockout samples were subjected to SDS-PAGE. Ab133346 and ab18058 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1000 dilution and 1/30000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-ISG15 antibody [EPR3446] (ab133346)

**All lanes :** Anti-ISG15 antibody [EPR3446] (ab133346) at 1/2000 dilution

**Lane 1 :** Untreated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2: HeLa (Human cervix adenocarcinoma epithelial cell) treated with 10 ng/ml IFN- a1 for 16 hours whole cell lysate

Lysates/proteins at 20 µg per lane.

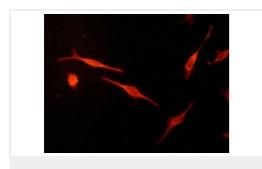
#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

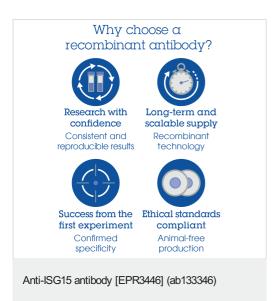
**Predicted band size:** 18 kDa **Observed band size:** 17 kDa

Exposure time: 3 minutes

Blocking/Diluting buffer and concentration: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-ISG15 antibody [EPR3446] (ab133346) ImmunImmunofluorescent analysis of ISG15 in HeLa cells stained with ab133346 at a 1/100 dilution.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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