abcam

ab315281

# Anti-ISG15 antibody [EPR28318-62]

Recombinant | RabMAb | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | EPR28318-62 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.529 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

### IHC-P

#### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/2000 |
| **Notes** | - |

#### Not recommended

| | |
|---|---|
| **Species** | Human, Rat |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/2000 |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Human, Rat |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/5000 |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Human, Rat |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/1000 |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Human, Rat |
| **Dilution info** | - |
| **Notes** | - |

## IP

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/30 |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Human, Rat |
| **Dilution info** | - |
| **Notes** | - |

## IHC-Fr

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/500 |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Human, Rat |
| **Dilution info** | - |

| | |
|---|---|
| **Notes** | - |

## Target data

See full target information Isg15

| | |
|---|---|
| **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. 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. Its target proteins include SERPINA3G/SPI2A, JAK1, MAPK3/ERK1, PLCG1, TRIM25, STAT5A, MAPK1/ERK2 and globin. 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. Can also isgylate: RIGI which inhibits its function in antiviral signaling response, IRF3 which inhibits its ubiquitination and degradation as well as EIF4E2 which enhances its cap structure-binding activity and translation-inhibition activity. Exhibits antiviral activity towards both DNA and RNA viruses, including influenza A and B virus, sindbis virus (SV) and herpes simplex type-1 (HHV-1). Plays a significant role in the control of neonatal Chikungunya virus (CHIKV) infection by acting as a putative immunomodulator of pro-inflammatory cytokines. Protects mice against the consequences of Chikungunya virus infection by down-regulating the pathogenic cytokine response, often denoted as the cytokine storm. Plays a role in erythroid differentiation. 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 (By similarity). |

## Storage

| | |
|---|---|
| **Shipped at conditions** | Conditional Ambient |
| **Appropriate short-term storage duration** | 1-2 weeks |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

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

**What are the advantages of a recombinant monoclonal antibody?**
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 batch production

For more information, read more on recombinant antibodies.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | ISG15 also known as G1P2 is a ubiquitin-like protein with a molecular weight of approximately 17 kDa. This protein gets expressed highly in response to interferon stimulation particularly in white blood cells such as macrophages and monocytes. It consists of two ubiquitin-like domains contributing to its functionality. ISG15 performs its mechanical role by adding itself to target proteins in a process known as ISGylation. This process resembles ubiquitination and serves to modify the functions of targeted proteins. |
| **Biological function summary** | ISG15 plays an important role in the immune response to viral infections. It acts as an antiviral agent by inhibiting viral replication. It interacts with and becomes a part of protein complexes modifying cellular proteins involved in immune regulation. ISG15 influences the stability function and localization of these target proteins enhancing antiviral defenses and potentially modulating immune signaling pathways. |
| **Pathways** | ISG15 operates within the interferon signaling pathway and the antiviral response pathway. In the interferon signaling pathway ISG15 interacts with proteins like UBE1L and UBE2L6 which act together for effective ISGylation of targets. In the antiviral response pathway ISG15 boosts the effects of proteins like IRF3 and STAT1 enhancing antiviral responses. These interactions allow ISG15 to effectively contribute to halting viral propagation. |
| **Associated diseases and disorders** | ISG15 connects to autoimmune diseases and certain viral infections. In autoimmune conditions like systemic lupus erythematosus abnormal ISG15 activity can contribute to disease pathology. ISG15 also interacts with proteins such as USP18 which regulates the expression of type I interferons to influence the disease processes. Furthermore ISG15 involvement in viral infections such as hepatitis C highlights its value in antiviral defenses. Understanding these interactions can support the development of therapies targeting ISG15 for managing immune-related conditions. |

# Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

We do not recommend this combination. It is not covered by our product promise.

---

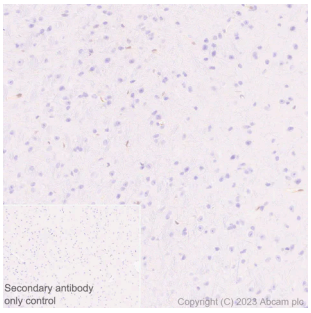
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

---

## 10 product images




### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ISG15 antibody [EPR28318-62] (ab315281)

ISG15 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse cererbrum using rabbit Anti-ISG15 antibody

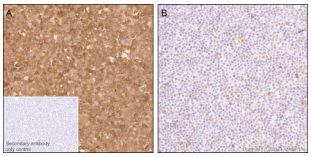
Immunohistochemical analysis of paraffin-embedded Mouse cererbrum tissue labeling ISG15 with ab315281 at 1/2000 (0.262 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Low expression: weak staining on mouse cerebrum. The section was incubated with ab315281 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

---



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ISG15 antibody [EPR28318-62] (ab315281)

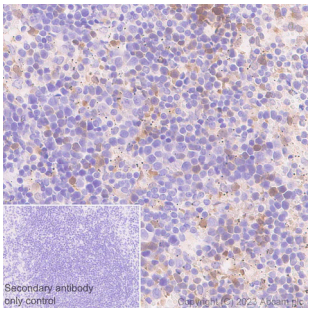
Immunohistochemical analysis of paraffin-embedded (A) RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) treated with 1 ug/ml Lipopolysaccharides (LPS) for 21 hours, 1 ug/ml Brefeldin A was then added for additional 3 hours (B) Untreated RAW 264.7 tissue labeling ISG15 with ab315281 at 1/2000 (0.262 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on (A) Raw 264.7 cell pellet treated with 1 ug/ml Lipopolysaccharides (LPS) for 21 hours, 1 ug/ml Brefeldin A was then added for additional 3 hours; weak staining on (B) Untreated RAW 264.7. The section was incubated with ab315281 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

---




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ISG15 antibody [EPR28318-62] (ab315281)

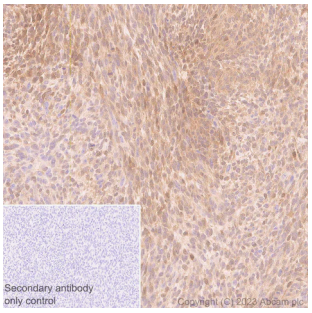
Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling ISG15 with ab315281 at 1/2000 (0.262 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on mouse spleen. The section was incubated with ab315281 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

---




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ISG15 antibody [EPR28318-62] (ab315281)

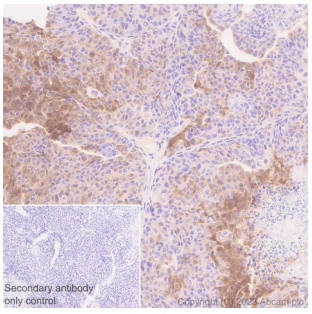
Immunohistochemical analysis of paraffin-embedded Mouse glioblastoma tissue labeling ISG15 with ab315281 at 1/2000 (0.262 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on mouse glioblastoma. The section was incubated with ab315281 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

---




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ISG15 antibody [EPR28318-62] (ab315281)

ISG15 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse breast cancer using rabbit Anti-ISG15 antibody

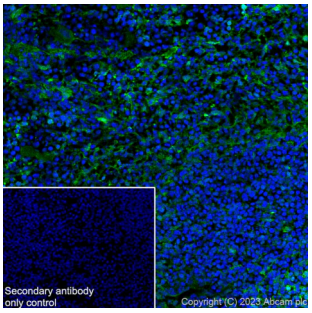
Immunohistochemical analysis of paraffin-embedded Mouse breast cancer tissue labeling ISG15 with ab315281 at 1/2000 (0.262 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on mouse breast cancer. The section was incubated with ab315281 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

---

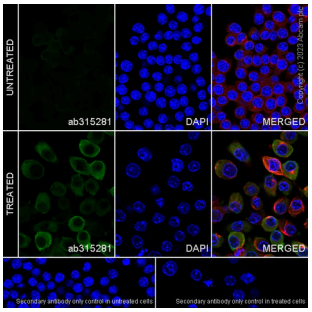



## Immunohistochemistry (Frozen sections) - Anti-ISG15 antibody [EPR28318-62] (ab315281)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse spleen (fresh frozen) tissue labeling ISG15 with ab315281 at 1/500 (1.048 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution (Green).

Confocal image showing positive staining on Mousee spleen. The nuclear counterstain was DAPI (Blue). The section was incubated with ab315281 for 60 mins at room temperature. The section was then mounted using Fluoromount®. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution.




## Immunocytochemistry/ Immunofluorescence - Anti-ISG15 antibody [EPR28318-62] (ab315281)

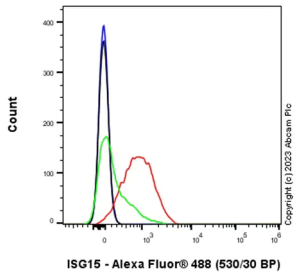
ISG15 Immunocytochemistry/ Immunofluorescence staining of Raw 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) using rabbit Anti-ISG15 antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Raw 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling ISG15 with ab315281 at 1/2000 (0.262 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green).

Confocal image showing increased cytoplasmic staining in RAW 264.7 cells treated with Lipopolysaccharide (LPS, 1 µg/mL) for 24 hours, then add Brefeldin A (BFA, 1 µg/mL) for 3 hours. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

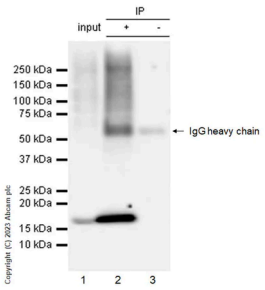
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.




## Flow Cytometry (Intracellular) - Anti-ISG15 antibody [EPR28318-62] (ab315281)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 1 µg/mL LPS for 24h and 1ug/ml BFA for 3 h (Red) / untreated RAW 264.7 (Green) cells labelling ISG15 with ab315281 at 1/5000 dilution (0.01 ug, Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.




## Immunoprecipitation - Anti-ISG15 antibody [EPR28318-62] (ab315281)

ISG15 was immunoprecipitated from 0.35 mg RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) treated with 1 ug/ml Lipopolysaccharides (LPS) for 21 hours, 1 ug/ml Brefeldin A was then added for additional 3 hours whole cell lysate with ab315281 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab315281 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) treated with 1 ug/ml Lipopolysaccharides (LPS) for 21 hours, 1 ug/ml Brefeldin A was then added for additional 3 hours whole cell lysate

Lane 2: ab315281 IP in RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) treated with 1 ug/ml Lipopolysaccharides (LPS) for 21 hours, 1 ug/ml Brefeldin A was then added for additional 3 hours whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab315281 in RAW 264.7 treated with 1 ug/ml Lipopolysaccharides (LPS) for 21 hours, 1 ug/ml Brefeldin A was then added for additional 3 hours whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 16815975).

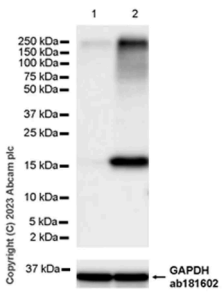
All lanes:
Immunoprecipitation - Anti-ISG15 antibody [EPR28318-62] (ab315281) at 1/30 dilution

All lanes:
RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) treated with 1 ug/ml Lipopolysaccharides (LPS) for 21 hours, 1 ug/ml Brefeldin A was then added for additional 3 hours whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 10s




## Western blot - Anti-ISG15 antibody [EPR28318-62] (ab315281)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The expression of ISG15 is upregulated in response to Lipopolysaccharides treatment (PMID: 11854279).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

All lanes:
Western blot - Anti-ISG15 antibody [EPR28318-62] (ab315281) at 1/1000 dilution

Lane 1:
Untreated RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 2:

RAW 264.7 treated with 1 µg/ml Lipopolysaccharides (LPS) for 21 hours, 1 µg/ml Brefeldin A was then added for additional 3 hours whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 17 kDa, 36 kDa

Exposure time: 10s

---

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.