abcam

Product datasheet

Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free ab234904

KO VALIDATED Recombinant RabMAb

2 Abreviews 9 Images

Overview

Product name	Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free
Description	Rabbit monoclonal [EPR21057-141] to STAT1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIP, WB, IHC-P, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HEK-293T and MCF7 cell lysate; Human heart tissue lysate. IHC-P: Human tonsil and kidney tissue; Paried human endometrial cancer and non-tumour endometrium tissue. Flow Cyt (intra): HeLa and MCF7 cells. IP: MCF7 whole cell lysate. ChIP1: HeLa cells.
General notes	ab234904 is the carrier-free version of **ab234400**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR21057-141
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab234904 in the following tested applications.

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

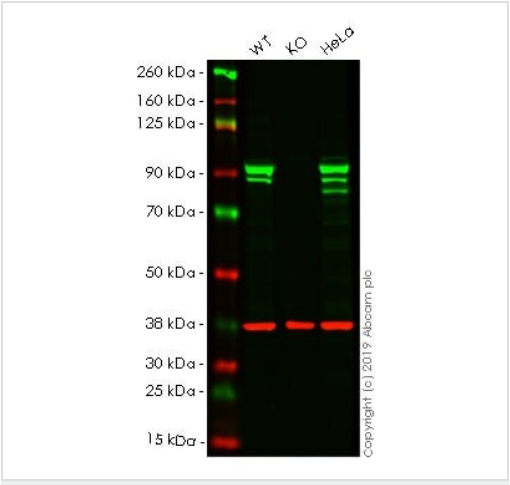
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 87 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.

Target

Function Signal transducer and activator of transcription that mediates signaling by interferons (IFNs). Following type I IFN (IFN-alpha and IFN-beta) binding to cell surface receptors, Jak kinases (TYK2 and JAK1) are activated, leading to tyrosine phosphorylation of STAT1 and STAT2. The phosphorylated STATs dimerize, associate with ISGF3G/IRF-9 to form a complex termed ISGF3 transcription factor, that enters the nucleus. ISGF3 binds to the IFN stimulated response element (ISRE) to activate the transcription of interferon stimulated genes, which drive the cell in an antiviral state. In response to type II IFN (IFN-gamma), STAT1 is tyrosine- and serine-phosphorylated. It then forms a homodimer termed IFN-gamma-activated factor (GAF), migrates into the nucleus and binds to the IFN gamma activated sequence (GAS) to drive the expression of the target genes, inducing a cellular antiviral state.

Involvement in disease	Note=STAT1 deficiency results in impaired immune response leading to severe mycobacterial and viral diseases. In the case of complete deficiency, patients can die of viral disease. Defects in STAT1 are a cause of mendelian susceptibility to mycobacterial disease (MSMD) [MIM:209950]; also known as familial disseminated atypical mycobacterial infection. This rare condition confers predisposition to illness caused by moderately virulent mycobacterial species, such as Bacillus Calmette-Guerin (BCG) vaccine and environmental non-tuberculous mycobacteria, and by the more virulent Mycobacterium tuberculosis. Other microorganisms rarely cause severe clinical disease in individuals with susceptibility to mycobacterial infections, with the exception of Salmonella which infects less than 50% of these individuals. The pathogenic mechanism underlying MSMD is the impairment of interferon-gamma mediated immunity whose severity determines the clinical outcome. Some patients die of overwhelming mycobacterial disease with lepromatous-like lesions in early childhood, whereas others develop, later in life, disseminated but curable infections with tuberculoid granulomas. MSMD is a genetically heterogeneous disease with autosomal recessive, autosomal dominant or X-linked inheritance.
Sequence similarities	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
Post-translational modifications	Phosphorylated on tyrosine and serine residues in response to IFN-alpha, IFN-gamma, PDGF and EGF. Phosphorylation on Tyr-701 (lacking in beta form) by JAK promotes dimerization and subsequent translocation to the nucleus. Phosphorylation on Ser-727 by several kinases including MAPK14, ERK1/2 and CAMKII on IFN-gamma stimulation, regulates STAT1 transcriptional activity. Phosphorylation on Ser-727 promotes sumoylation though increasing interaction with PIAS. Phosphorylation on Ser-727 by PKCdelta induces apoptosis in response to DNA-damaging agents. Sumoylated by SUMO1, SUMO2 and SUMO3. Sumoylation is enhanced by IFN-gamma-induced phosphorylation on Ser-727, and by interaction with PIAS proteins. Enhances the transactivation activity. ISGylated.
Cellular localization	Cytoplasm. Nucleus. Translocated into the nucleus in response to IFN-gamma-induced tyrosine phosphorylation and dimerization.

Images



Western blot - Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free (ab234904)

All lanes : Anti-STAT1 antibody [EPR21057-141] - ChIP Grade (**ab234400**) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate

Lane 2 : STA1 knockout HAP1 whole cell lysate

Lane 3 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

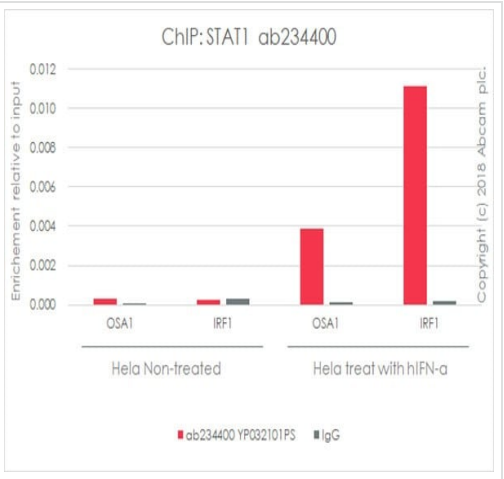
Predicted band size: 87 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (**ab234400**).

Lanes 1 - 3: Merged signal (red and green). Green - **ab234400** observed at 87 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

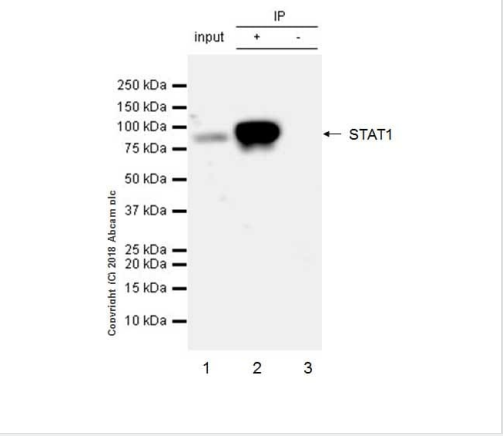
ab234400 was shown to specifically react with STAT1 in wild-type HAP1 cells as signal was lost in STA1 knockout cells. Wild-type and STA1 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab234400 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 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.



ChIP - Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free (ab234904)

Chromatin was prepared from HeLa (starve overnight) + hIFN-α (serum-starved overnight) (1,000 units/ml,30 min) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 2 µg of **ab234400** (red), and 20 µl of protein A/G sepharose beads slurry (10 µl of sepharose A beads + 10 µl of sepharose G beads). 2 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (SYBR approach).
ChIP results are consistent with the literature (PMID: 16319195).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab234400**).



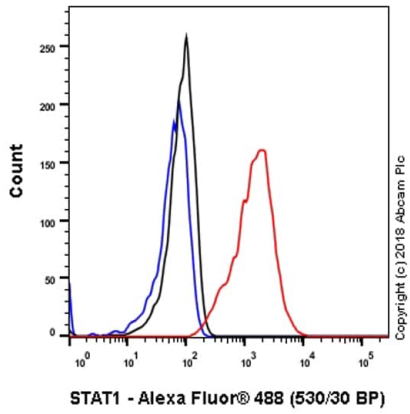
Immunoprecipitation - Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free (ab234904)

STAT1 was immunoprecipitated from 0.35 mg of MCF7 (human breast adenocarcinoma cell line) whole cell lysate with **ab234400** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab234400** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1/5000 dilution.
Lane 1: MCF7 whole cell lysate 10 µg (input).
Lane 2: **ab234400** IP in MCF7 whole cell lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab234400** in MCF7 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 3 minutes.

The doublet observed in some lanes likely represent the α and β isoforms of STAT1 (PMID: 8647800).

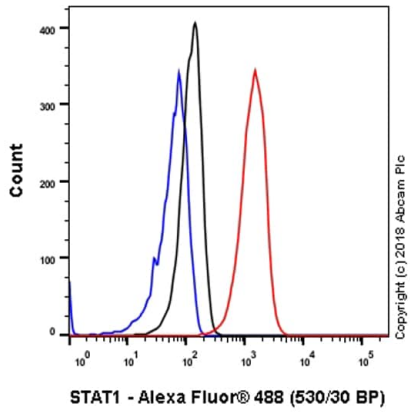
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab234400**).



Flow Cytometry (Intracellular) - Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free (ab234904)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized MCF7 (human breast adenocarcinoma cell line) cells labeling STAT1 with **ab234400** at 1/500 (Red) compared with Rabbit monoclonal IgG (**ab172730**) (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

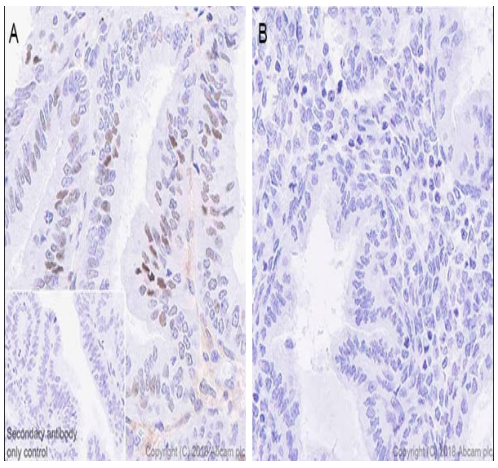
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab234400**).



Flow Cytometry (Intracellular) - Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free (ab234904)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling STAT1 with **ab234400** at 1/500 (Red) compared with Rabbit monoclonal IgG (**ab172730**) (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab234400**).



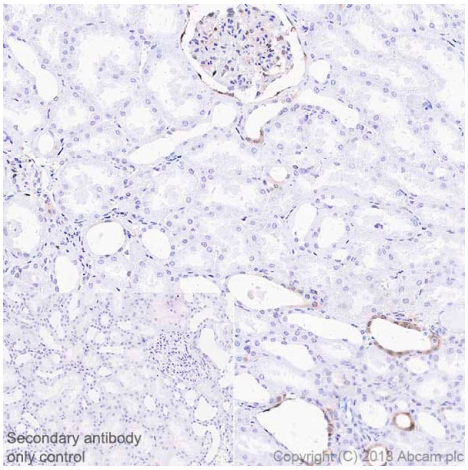
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free (ab234904)

Immunohistochemical analysis of paraffin-embedded paired human endometrial cancer **(A)** and non-tumor endometrium tissue **(B)** labeling STAT1 with **ab234400** at 1/5000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Much higher staining intensity of endometrial cancer **(A)** than its paired non-tumor endometrium **(B)** (PMID: 25267067) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab234400**).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



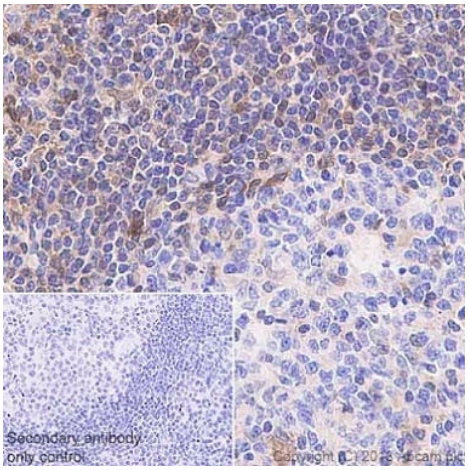
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free (ab234904)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling STAT1 with **ab234400** at 1/5000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Positive staining on glomerulus and some renal tubules of human kidney (PMID: 26678048) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab234400**).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



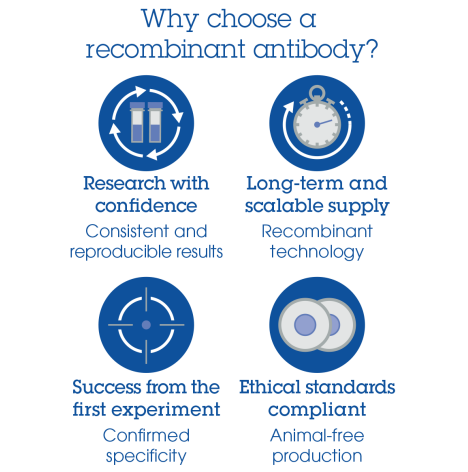
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free (ab234904)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling STAT1 with **ab234400** at 1/5000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Positive staining mainly on interfollicular cells of human tonsil (PMID: 25336386; PMID: 25921060) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab234400**).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-STAT1 antibody [EPR21057-141] - BSA and Azide free (ab234904)

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