# abcam

## Product datasheet

# Anti-STING antibody [EPR13130-55] - BSA and Azide free ab242019



Recombinant

RabMAb

# 9 Images

#### Overview

Product name Anti-STING antibody [EPR13130-55] - BSA and Azide free

**Description**Rabbit monoclonal [EPR13130-55] to STING - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), IP, ICC/IF, IHC-P, WB

Species reactivity Reacts with: Human

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Wild-type THP-1 cell lysate. IHC-P: Human tonsil, colon cancer and prostate hyperplasia

tissues. ICC/IF: THP-1 cells. Flow Cyt (intra): THP-1 cells. IP: THP-1 whole cell lysate.

**General notes** ab242019 is the carrier-free version of <u>ab239074</u>.

Our <u>carrier-free</u> 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

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### **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

Clone number Monoclonal EPR13130-55

**Isotype** IgG

### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab242019 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)		1/500.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
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.
WB		Use at an assay dependent concentration. Detects a band of approximately 36 kDa (predicted molecular weight: 42 kDa).

# **Target**

**Function** Facilitator of innate immune signaling that promotes the production of type I interferon (IFN-alpha

and IFN-beta). Innate immune response is triggered in response to non-CpG double-stranded DNA from viruses and bacteria delivered to the cytoplasm. Able to activate both NF-kappa-B and IRF3 transcription pathways to induce expression of type I interferon and exert a potent anti-viral state following expression. May be involved in translocon function, the translocon possibly being able to influence the induction of type I interferons. May be involved in transduction of apoptotic signals via its association with the major histocompatibility complex class II (MHC-II). Mediates death signaling via activation of the extracellular signal-regulated kinase (ERK) pathway.

**Tissue specificity** Ubiquitously expressed.

**Sequence similarities** Belongs to the TMEM173 family.

Post-translational Phosphorylated on tyrosine residues upon MHC-II aggregation (By similarity). Phosphorylated on

#### modifications

Ser-358 by TBK1, leading to activation and production of IFN-beta.

Ubiquitinated. 'Lys-63'-linked ubiquitination mediated by TRIM56 at Lys-150 promotes

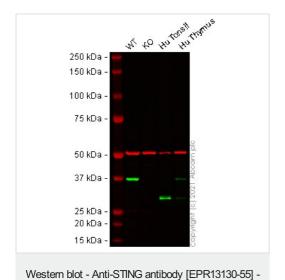
homodimerization and recruitment of the antiviral kinase TBK1 and subsequent production of IFN-beta. 'Lys-48'-linked polyubiquitination at Lys-150 occurring after viral infection is mediated by

RNF5 and leads to proteasomal degradation.

#### **Cellular localization**

Endoplasmic reticulum membrane. Mitochondrion outer membrane. Cell membrane. Cytoplasm > perinuclear region. In response to double-stranded DNA stimulation, relocalizes to perinuclear region, where the kinase TBK1 is recruited.

#### **Images**



BSA and Azide free (ab242019)

**All lanes :** Anti-STING antibody [EPR13130-55] (ab239074) at 1/1000 dilution

Lane 1: Wild-type THP-1 cell lysate

Lane 2: TMEM173 knockout THP-1 cell lysate

Lane 3: Human Tonsil tissue lysate

Lane 4: Human Thymus tissue lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

different buffer formulation (ab239074).

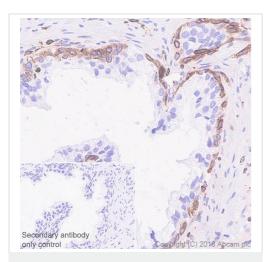
Predicted band size: 42 kDa
Observed band size: 37 kDa

This data was developed using the same antibody clone in a

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab239074</u> observed at 37 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab239074 was shown to react with TMEM173 in wild-type THP-1 cells in Western blot with loss of signal observed in TMEM173 knockout cell line ab270493 (TMEM173 knockout cell lysae ab270516). Wild-type THP-1 and TMEM173 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab239074 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776)

secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STING antibody

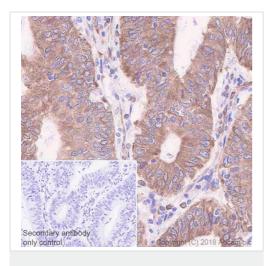
[EPR13130-55] - BSA and Azide free (ab242019)

Immunohistochemical analysis of paraffin-embedded human prostate hyperplasia tissue labeling TMEM173 with <a href="mailto:ab239074">ab239074</a> at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining in basal cells of human prostate hyperplasia is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG 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 (ab239074).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STING antibody

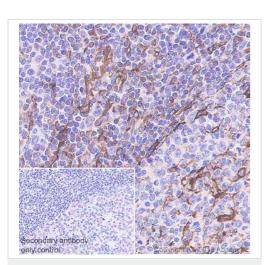
[EPR13130-55] - BSA and Azide free (ab242019)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling TMEM173 with <u>ab239074</u> at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining in human colon cancer (PMID: 26748708). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG 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 (ab239074).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STING antibody

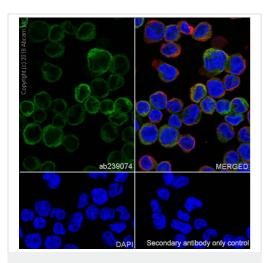
[EPR13130-55] - BSA and Azide free (ab242019)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling TMEM173 with **ab239074** at 1/4000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Positive staining in human tonsil (PMID:28874664). 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 (ab239074).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



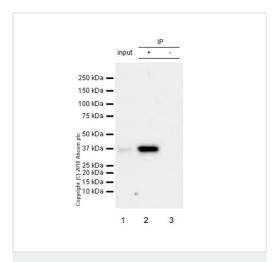
Immunocytochemistry/ Immunofluorescence - Anti-STING antibody [EPR13130-55] - BSA and Azide free (ab242019)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized THP-1 (human monocytic leukemia cell line) cells labeling TMEM173 with <a href="mailto:ab239074">ab239074</a> at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (<a href="mailto:ab150077">ab150077</a>) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic and membranous staining in THP-1 cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (**ab195889**) (red) at 1/200 dilution.

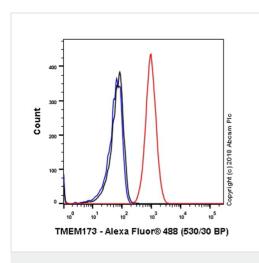
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239074).



Immunoprecipitation - Anti-STING antibody

[EPR13130-55] - BSA and Azide free (ab242019)



Flow Cytometry (Intracellular) - Anti-STING antibody [EPR13130-55] - BSA and Azide free (ab242019)

TMEM173 was immunoprecipitated from 0.35 mg of THP-1 (human monocytic leukemia cell line) whole cell lysate with <u>ab239074</u> at 1/30 dilution. Western blot was performed from the immunoprecipitate using <u>ab239074</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/1000 dilution.

Lane 1: THP-1 whole cell lysate 10 µg (Input).

Lane 2: ab239074 IP in THP-1 whole cell lysate.

Lane 3: Rabbit monoclonal  $\lg G$  ( $\underline{ab172730}$ ) instead of  $\underline{ab239074}$  in THP-1 whole cell lysate.

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

Exposure time: 30 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239074).

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized THP-1 (human monocytic leukemia cell line) cell line labeling TMEM173 with <u>ab239074</u> at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>) 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 (ab239074).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STING antibody

[EPR13130-55] - BSA and Azide free (ab242019)

Tissue Microarrays stained for "Anti-STING antibody [EPR13130-55]" using "ab239074" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab239074 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



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