abcam

Product datasheet

Anti-STING antibody [EPR13130] - BSA and Azide free ab227128

KO VALIDATED Recombinant RabMAb

10 Images

Overview

Product name	Anti-STING antibody [EPR13130] - BSA and Azide free
Description	Rabbit monoclonal [EPR13130] to STING - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	ICC/IF: HeLa cells. WB: Wild-type THP-1,Human Thymus cell lysate
General notes	ab227128 is the carrier-free version of **ab181125**. 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. 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	EPR13130
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227128 in the following tested applications.

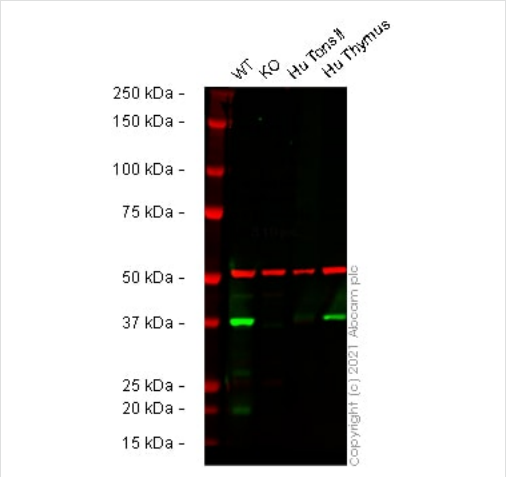
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 42 kDa.
ICC/IF		Use at an assay dependent concentration.

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



Western blot - Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

All lanes : Anti-STING antibody [EPR13130] (**ab181125**) at 1/1000 dilution

Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : TMEM173 knockout THP-1 cell lysate
Lane 3 : Human Tonsil cell lysate
Lane 4 : Human Thymus cell lysate

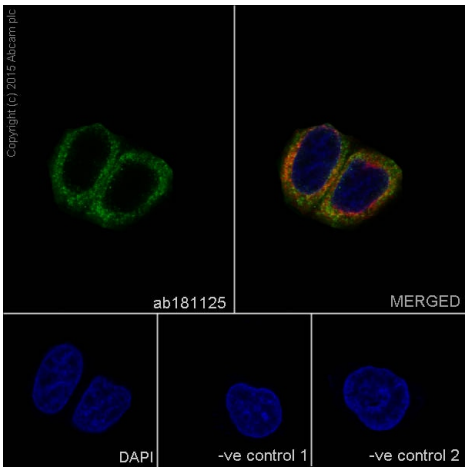
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

False colour image of Western blot: Anti-STING antibody [EPR13130] staining at 1/1000 dilution, shown in green; loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) at 1/20000 dilution, shown in red. In Western blot, **ab181125** was shown to bind specifically to STING. A band was observed at 37 kDa in wild-type THP-1 cell lysates with no signal observed at this size in TMEM173 knockout cell line **ab270493** (knockout cell lysate **ab270516**). To generate this image, wild-type and TMEM173 knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.

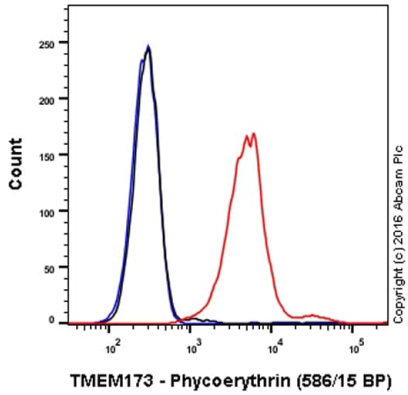
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181125**).



Immunocytochemistry/ Immunofluorescence - Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

Immunofluorescence staining of HeLa cells with purified **ab181125** at a working dilution of 1/1000, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified **ab181125** was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.

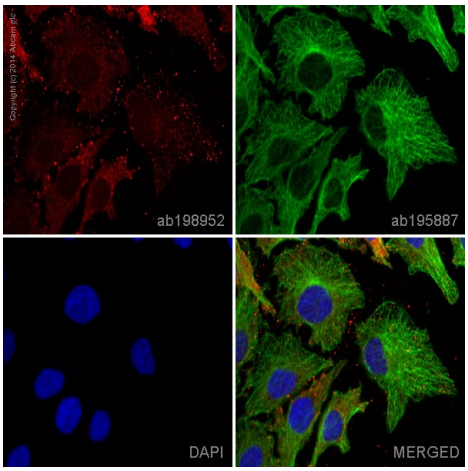
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181125**).



Flow Cytometry (Intracellular) - Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

Clone EPR13130 (ab227128) has been successfully conjugated by Abcam. This image was generated using Anti-TMEM173 antibody [EPR13130] (PE). Please refer to **ab208874** for protocol details.

Overlay histogram showing HeLa cells stained with **ab208874** (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (**ab208874**, 1/500 dilution) for 30 min at 22°C.Isotype control antibody (black line) was rabbit IgG (monoclonal) Phycoerythrin (**ab209478**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter. This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



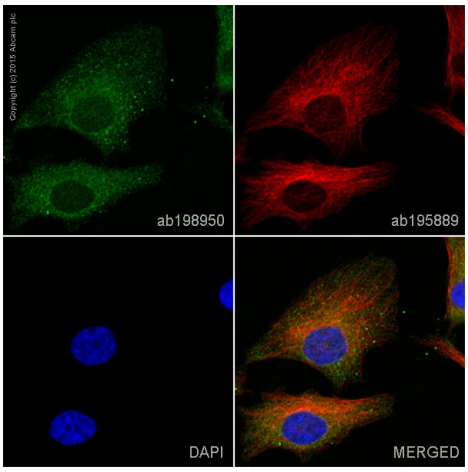
Immunocytochemistry/ Immunofluorescence - Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

Clone EPR13130 (ab227128) has been successfully conjugated by Abcam. This image was generated using Anti-TMEM173 antibody [EPR13130] (Alexa Fluor® 647). Please refer to **ab198952** for protocol details.

ab198952 staining TMEM173 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab198952** at 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in HeLacells fixed with 4% formaldehyde (10 min).

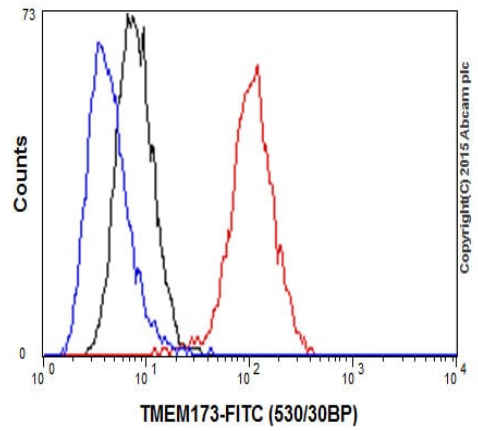


Immunocytochemistry/ Immunofluorescence - Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

Clone EPR13130 (ab227128) has been successfully conjugated by Abcam. This image was generated using Anti-TMEM173 antibody [EPR13130] (Alexa Fluor® 488). Please refer to **ab198950** for protocol details.

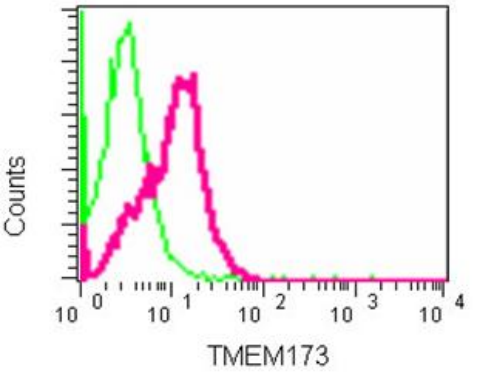
ab198950 staining TMEM173 in HeLa cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab198950** at 1/200 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

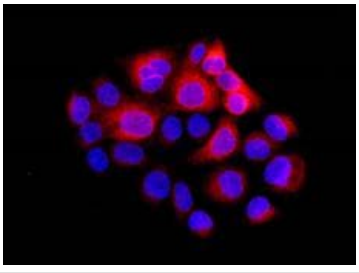
Overlay histogram showing THP-1 cells fixed in 4% PFA and stained with purified **ab181125** at a dilution of 1 in 20 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181125**).



Flow Cytometry (Intracellular) - Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

Intracellular Flow Cytometry analysis of THP1 cells using unpurified **ab181125** at a 1/10 dilution (red) or a Rabbit monoclonal IgG (negative) (green). Goat anti rabbit IgG (FITC) secondary used at a 1/150 dilution.

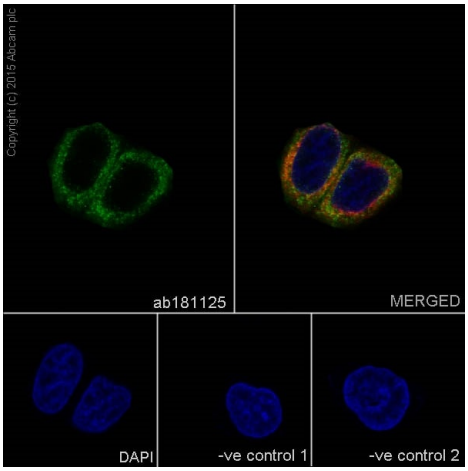
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181125**).



Immunocytochemistry/ Immunofluorescence - Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

Immunofluorescence analysis of HACAT cells (fixative 4% paraformaldehyde) labeling TMEM173 with unpurified **ab181125** at a 1/100 dilution, and counterstained with DAPI. Goat anti rabbit IgG (Dylight® 555) secondary used at a 1/200 diution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181125**).



Immunocytochemistry/ Immunofluorescence - Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

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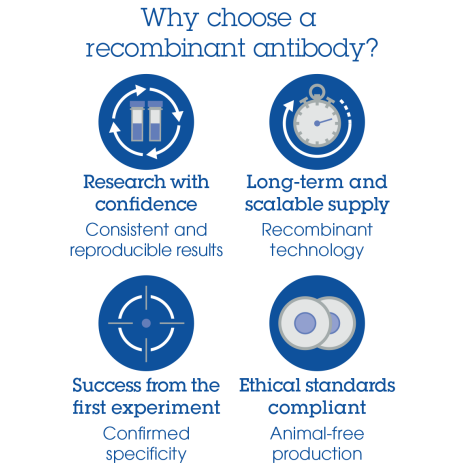

Anti-STING antibody [EPR13130] - BSA and Azide free (ab227128)

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