abcam

Product datasheet

Anti-MTAP antibody [EPR6893] - BSA and Azide free ab232417



Recombinant

RabMAb

9 Images

Overview

Product name Anti-MTAP antibody [EPR6893] - BSA and Azide free

Description Rabbit monoclonal [EPR6893] to MTAP - BSA and Azide free

Host species Rabbit

Specificity The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

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

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HeLa, 293T, HT29, C6, RAW 264.7, and NIH 3T3 cell lysates. ICC/IF: HeLa cells. Flow Cyt

(intra): HeLa cells. IHC-P: Human kidney, mouse kidney, and human lung carcinoma tissue.

General notes ab232417 is the carrier-free version of **ab126770**.

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[®] 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.

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Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to ${\hbox{\bf RabMAb}}^{\hbox{\bf @}}$ 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 EPR6893

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab232417 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		Use at an assay dependent concentration. Detects a band of approximately 29 kDa (predicted molecular weight: 31 kDa).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Target

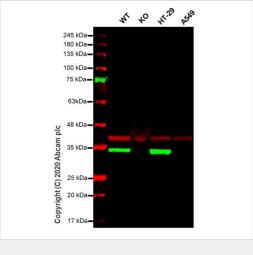
Function Plays a major role in polyamine metabolism and is important for the salvage of both adenine and

methionine.

Tissue specificity Ubiquitously expressed.

Sequence similaritiesBelongs to the PNP/MTAP phosphorylase family.

Cellular localization Cytoplasm.



Western blot - Anti-MTAP antibody [EPR6893] - BSA and Azide free (ab232417)

All lanes : Anti-MTAP antibody [EPR6893] (**ab126770**) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: MTAP knockout HeLa cell lysate

Lane 3 : HT-29 cell lysate Lane 4 : A549 cell lysate

Lysates/proteins at 20 µg per lane.

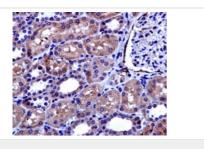
Performed under reducing conditions.

Predicted band size: 31 kDa
Observed band size: 32 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab126770</u>).

anes 1-4: Merged signal (red and green). Green - <u>ab126770</u> observed at 32 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) observed at 37 kDa.

ab126770 Anti-MTAP antibody [EPR6893] was shown to specifically react with MTAP in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265272 (knockout cell lysate ab257194) was used. Wild-type and MTAP knockout samples were subjected to SDS-PAGE. ab126770 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



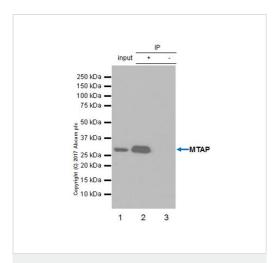
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MTAP antibody

[EPR6893] - BSA and Azide free (ab232417)

Formalin-fixed, paraffin-embedded human kidney tissue stained for MTAP with unpurified <u>ab126770</u> (1/50 dilution) in immunohistochemical analysis.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab126770</u>).

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunoprecipitation - Anti-MTAP antibody

[EPR6893] - BSA and Azide free (ab232417)

ab126770 (purified) at 1:50 dilution (2μg) immunoprecipitating MTAP in HT-29 whole cell lysate.

Lane 1 (input): HT-29 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate 10ug

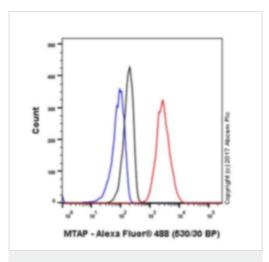
Lane 2 (+): ab126770 & HT-29 whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab126770 in HT-29 whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.

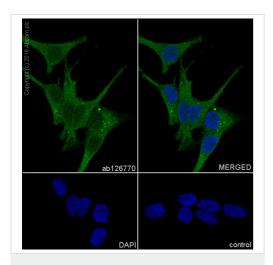
Blocking and diluting buffer: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab126770</u>).

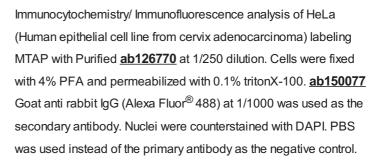


Flow Cytometry (Intracellular) - Anti-MTAP antibody [EPR6893] - BSA and Azide free (ab232417)

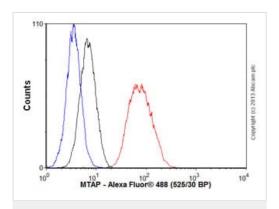
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling MTAP with purified ab126770 at 1/90 dilution (10 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit lgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab126770).



Immunocytochemistry/ Immunofluorescence - Anti-MTAP antibody [EPR6893] - BSA and Azide free (ab232417)



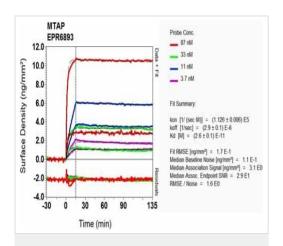
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab126770).



Flow Cytometry (Intracellular) - Anti-MTAP antibody [EPR6893] - BSA and Azide free (ab232417)

Overlay histogram showing HeLa cells stained with unpurified ab126770 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab126770, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab126770).

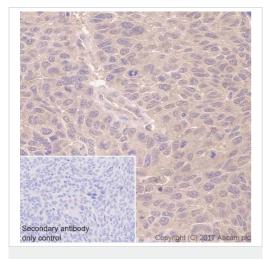


Ol-RD Scanning - Anti-MTAP antibody [EPR6893] - BSA and Azide free (ab232417)

Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about KD

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab126770).

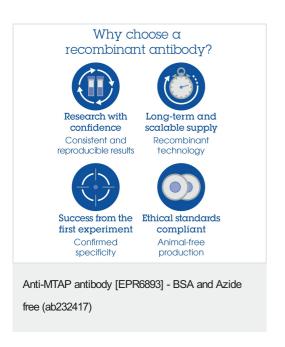


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MTAP antibody

[EPR6893] - BSA and Azide free (ab232417)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human lung carcinoma tissue sections labeling MTAP with Purified <u>ab126770</u> at 1:1000 dilution (0.89 µg/ml). Heat mediated antigen retrieval was performed using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab126770</u>).



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