abcam

Product datasheet

Anti-B MyB (phospho T487) antibody [EPR2204Y] ab76009



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Overview

Product name Anti-B MyB (phospho T487) antibody [EPR2204Y]

Description Rabbit monoclonal [EPR2204Y] to B MyB (phospho T487)

Host species Rabbit

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

Species reactivity Reacts with: Human

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

Positive control WB: 293T transfected with human B MyB (WT) expression vector containing a myc-His-tag®,

whole cell lysate and HeLa nuclear fraction lysate. ICC/IF: HeLa cells. IHC-P: Human lung and

colon cancer tissues. Flow Cyt (Intra): HeLa cells.

General notesThis 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

Purity Protein A purified

Clonality Monoclonal

1

Clone number EPR2204Y

Isotype IgG

Applications

The Abpromise guarantee Our Abp

Our $\underline{\textbf{Abpromise guarantee}}$ covers the use of ab76009 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 |
|------------------|-----------------|--|
| Dot blot | | 1/1000. |
| Flow Cyt (Intra) | | 1/1000. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody. |
| ICC/IF | | 1/1000. |
| WB | | 1/1000. Predicted molecular weight: 79 kDa. |
| IHC-P | **** <u>(1)</u> | 1/8000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

Target

Function Transcription factor involved in the regulation of cell survival, proliferation, and differentiation.

Transactivates the expression of the CLU gene.

Sequence similaritiesContains 3 HTH myb-type DNA-binding domains.

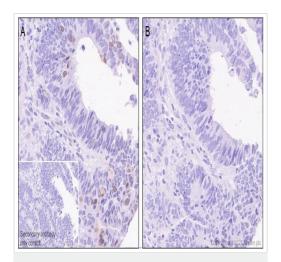
Post-translational modifications

Phosphorylated by cyclin A/CDK2 during S-phase. Phosphorylation at Thr-520 is probably

involved in transcriptional activity.

Cellular localization Nucleus.

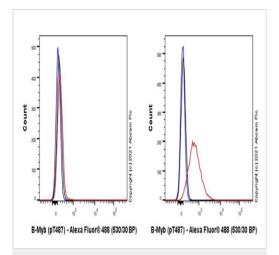
Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-B MyB (phospho T487) antibody [EPR2204Y] (ab76009)

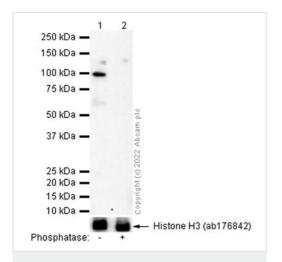
Immunohistochemistry analysis of paraffin-embedded Human colon cancer tissue sections labelling B MyB (phospho T487) with ab76009 at 1/8000 dilution. The section was incubated with ab76009 for 30 mins at room temperature. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

Nuclear staining on Human colon cancer tissue without alkaline phosphatase treatment (Image A); No signal was detected when tissues were treated with alkaline phosphatase (Image B). The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument.



Flow Cytometry (Intracellular) - Anti-B MyB (phospho T487) antibody [EPR2204Y] (ab76009)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells treated with Alkaline Phosphatase overnight (Left) and untreated HeLa cells (Right) labeling B MyB (phospho T487) with ab76009 at 1/1000 dilution (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150081) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (ab172730) (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-B MyB (phospho T487) antibody [EPR2204Y] (ab76009)

All lanes : Anti-B MyB (phospho T487) antibody [EPR2204Y] (ab76009) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) nuclear fraction lysate

Lane 2 : HeLa nuclear fraction lysate treated with Alkaline Phosphatase for 1 hour

Lysates/proteins at 15 µg per lane.

Secondary

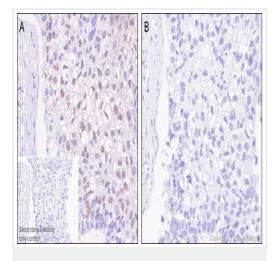
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 79 kDa Observed band size: 100 kDa

Exposure time: 120 seconds

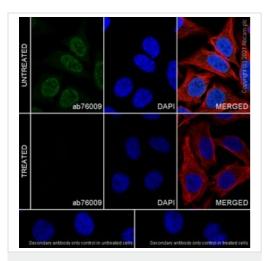
Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST



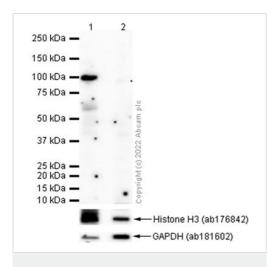
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-B MyB (phospho T487) antibody [EPR2204Y] (ab76009)

Immunohistochemistry analysis of paraffin-embedded Human lung cancer tissue sections labelling B MyB (phospho T487) with ab76009 at 1/8000 dilution. The section was incubated with ab76009 for 30 mins at room temperature. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins. Nuclear staining on Human lung cancer tissue without alkaline phosphatase treatment (Image A); No signal was detected when tissues were treated with alkaline phosphatase (Image B). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

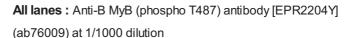


Immunocytochemistry/ Immunofluorescence - Anti-B MyB (phospho T487) antibody [EPR2204Y] (ab76009)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling B MyB (phospho T487) with primary antibody anti-B MyB (phospho T487) (ab76009) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody at 1/1000 dilution. Confocal image showing nuclear staining in HeLa cells and no staining in HeLa cells with Alkaline Phosphatase treatment 37 for 1 hour. Anti-alpha Tubulin antibody (DM1A) - Microtubule Marker (Alexa Fluor® 594) (ab195889) was used to counterstain tubulin at 1/200 dilution. The nuclear counter stain is DAPI (blue).



Western blot - Anti-B MyB (phospho T487) antibody [EPR2204Y] (ab76009)



Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) nuclear fraction lysate

Lane 2: HeLa (Human cervix adenocarcinoma epithelial cell) without nuclear fraction lysate

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 79 kDa **Observed band size:** 100 kDa

Exposure time: 60 seconds

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

All lanes : Anti-B MyB (phospho T487) antibody [EPR2204Y] (ab76009) at 1/1000 dilution

Lane 1: 293T (Human embryonic kidney epithelial cell) transfected with empty vector (vector control), containing a myc-His-tag®, whole cell lysate

Lane 2: 293T transfected with human B MyB (WT) expression vector containing a myc-His-tag®, whole cell lysate

Lysates/proteins at 20 µg per lane.

1 2 250 kDa — 150 kDa — 100 kDa — 75 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 10 kDa — 11 2 His tag (ab213204)

Western blot - Anti-B MyB (phospho T487) antibody [EPR2204Y] (ab76009)

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 79 kDa **Observed band size:** 100 kDa

Exposure time: 20 seconds

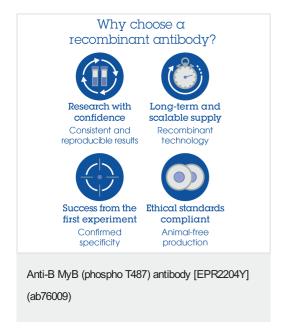
Blocking buffer and concentration: 5% NFDM/TBST Diluting buffer and concentration: 5% NFDM/TBST

Dot blot analysis of B MyB (pT487) phospho peptide (lane 1) and B MyB non-phospho peptide (lane 2) with ab76009 at a 1/1000 dilution. Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) was used as the secondary antibody at a dilution of 1/20,000.

Blocking and dilution buffer: 5% NFDM/TBST

Exposure time: 3 minutes





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