# abcam

### Product datasheet

## Anti-KIF5B+KIF5C antibody [EPR10276(B)] ab167429

KO VALIDATED Recombinant RabMAb

\*\*\*\*\* <u>3 Abreviews</u> <u>37 References</u> 11 Images

Overview

Product name	Anti-KIF5B+KIF5C antibody [EPR10276(B)]		
Description	Rabbit monoclonal [EPR10276(B)] to KIF5C + KIF5B		
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: Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP		
Species reactivity	Reacts with: Mouse, Rat, Human		
Immunogen	Synthetic peptide corresponding to Human KIF5B aa 1-100. Database link: <u>P33176</u>		
Positive control	Human fetal kidney, HeLa, Jurkat and HepG2 lysates; Human brain tissue; HeLa cells.		
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>		

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.21% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR10276(B)
lsotype	lgG

#### **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab167429 in the following tested applications.

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

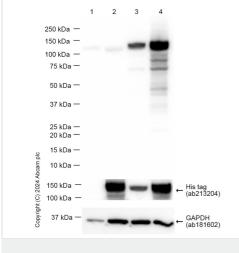
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB	$\star$ $\star$ $\star$ $\star$ $\star$ (2)	1/1000 - 1/10000. Predicted molecular weight: 110 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC 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		1/100 - 1/500.
IP		1/10 - 1/100.

#### Target

#### **Cellular localization**

KIF5C: Cytoplasm > cytoskeleton. KIF5B: Cytoplasm, cytoskeleton. Uniformly distributed between soma and neurites in hippocampal neurons.

#### Images





All lanes : Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429) at 1/1000 dilution Lane 1 : 293T (Human embryonic kidney epithelial cell) +OE-empty Lane 2 : 293T (Human embryonic kidney epithelial cell) +OEmouse KIF5A Lane 3 : 293T (Human embryonic kidney epithelial cell) +OEmouse KIF5C Lane 4 : 293T (Human embryonic kidney epithelial cell) +OEmouse KIF5B

Lysates/proteins at 20  $\mu g$  per lane.

#### Secondary

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

dilution

Predicted band size: 110 kDa Observed band size: 110 kDa

Exposure time: 1 second

Blocking and diluting buffer and concentration: 5% NFDM/TBST <u>ab181602</u> was used a GAPDH loading control.

All lanes : Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429) at 1/5000 dilution (purified)

Lane 1 : Jurkat (human acute T cell leukemia) whole cell lysates Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell ) whole cell lysates Lane 3 : Mouse brain lysates

Lane 4 : Rat brain lysates

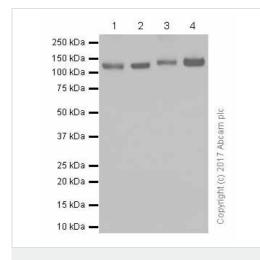
Lysates/proteins at 20 µg per lane.

#### Secondary

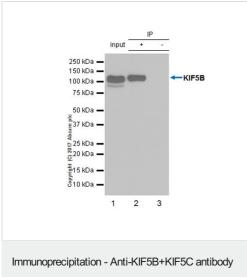
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 110 kDa

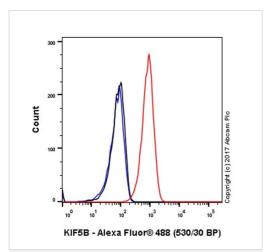
Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429)



[EPR10276(B)] (ab167429)

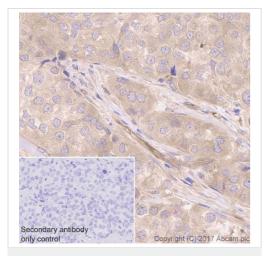


Flow Cytometry (Intracellular) - Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429) ab167429 (purified) at 1:20 dilution (2µg) immunoprecipitating KIF5B in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg Lane 2 (+): ab167429 & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab167429 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

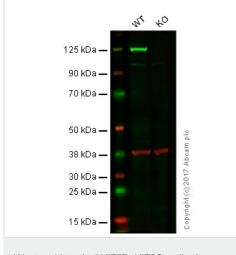
For western blotting, VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) was used for detection at 1:1000 dilution. Blocking and diluting buffer: 5% NFDM/TBST.

Intracellular Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling KIF5B with purified ab167429 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast carcinoma tissue sections labeling KIF5B with Purified ab167429 at 1:250 dilution (0.07 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (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.

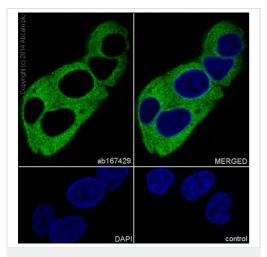
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429)



Western blot - Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg) Lane 2: KIF5B knockout HAP1 whole cell lysate (20 µg) Lanes 1 - 2: Merged signal (red and green). Green - ab167429 observed at 110 kDa. Red - loading control, <u>ab9484</u>, observed at 37 kDa.

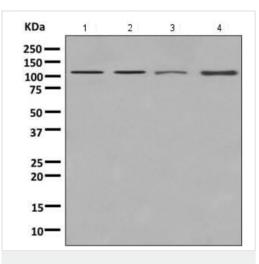
Unpurified ab167429 was shown to specifically react with KIF5B in wild-type cells as signal was lost in KIF5B knockout cells. Wild-type and KIF5B knockout samples were subjected to SDS-PAGE. Ab167429 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/Immunofluorescence analysis HepG2(human hepatocellular carcinoma) labelling KIF5B with purified ab167429 at 1/500. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. An Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Control: PBS only

Immunocytochemistry/ Immunofluorescence - Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429)



Western blot - Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429) **All lanes :** Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429) at 1/1000 dilution (unpurified)

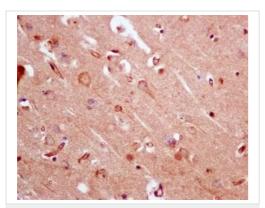
- Lane 1 : Human fetal kidney lysate Lane 2 : HeLa lysate Lane 3 : Jurkat lysate
- Lane 4 : HepG2 lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes : Goat anti-rabbit HRP at 1/2000 dilution

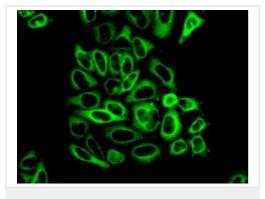
Predicted band size: 110 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429)

Immunohistochemical analysis of formalin-fixed, paraffin-embedded Human brain tissue, labeling KIF5B using unpurified ab167429 at a 1/100 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-KIF5B+KIF5C antibody [EPR10276(B)] (ab167429)



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Immunofluorescence analysis of HeLa cells, labeling KIF5B using unpurified ab167429 at a 1/100 dilution.