abcam

Product datasheet

Anti-Mark3 antibody [EPR633Y] ab52626

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Mark3 antibody [EPR633Y]
Description	Rabbit monoclonal [EPR633Y] to Mark3
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, Flow Cyt (Intra), ICC/IF **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide within Human Mark3 aa 600-700 (C terminal). The exact sequence is proprietary.
Positive control	WB: HeLa, K562 and NIH/3T3 cell lysate. ICC/IF: MCF-7 cells. Flow Cyt (intra): HeLa cells. IP: K562 cells.
General notes	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® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

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), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR633Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52626 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		1/1000 - 1/2000. Detects a band of approximately 86 kDa.
IP	★★★★★ (1)	1/20 - 1/60.
Flow Cyt (Intra)		1/30 - 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50 - 1/100.

Application notes Is unsuitable for IHC-P.

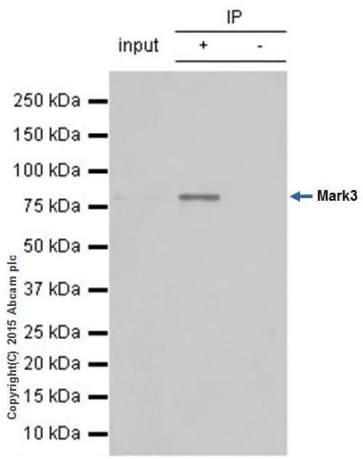
Target

Function Involved in the specific phosphorylation of microtubule-associated proteins for tau, MAP2 and MAP4. Phosphorylates CDC25C on 'Ser-216'.

Tissue specificity Ubiquitous.

Sequence similarities Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. MARK subfamily.
Contains 1 KA1 (kinase-associated) domain.
Contains 1 protein kinase domain.
Contains 1 UBA domain.

Images



Immunoprecipitation - Anti-Mark3 antibody [EPR633Y] (ab52626)

ab52626 (purified) at a dilution of 1/20 immunoprecipitating Mark3 in K562 whole cell lysate.

Lane 1 (input): K562 whole cell lysate (10µg)

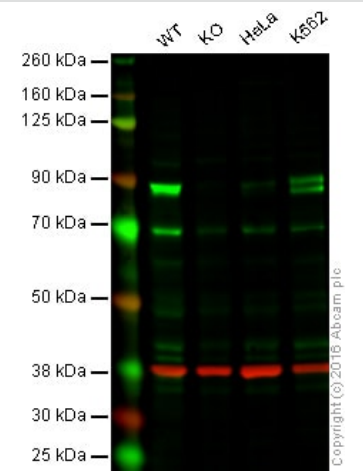
Lane 2 (+): ab52626 + K562 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab52626 in K562 whole cell lysate.

For western blotting, **ab131366** VeriBlot for IP (HRP) was used for detection (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

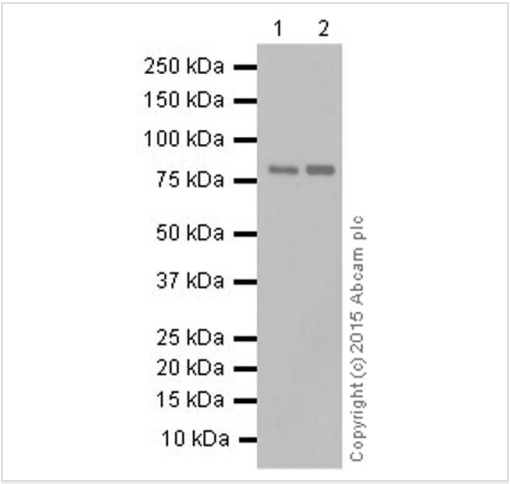
Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Mark3 antibody [EPR633Y] (ab52626)

Lane 1: Wild-type HAP1 cell lysate (40 µg)
Lane 2: MARK3 knockout HAP1 cell lysate (40 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: K562 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab52626 observed at 85 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab52626 was shown to recognize Mark3 when Mark3 knockout samples were used, along with additional cross-reactive bands. Wild-type and Mark3 knockout samples were subjected to SDS-PAGE. Ab52626 and **ab8245** (loading control to GAPDH) were diluted at 1/1000 and 1:10,000 dilution respectively and incubated overnight at 4C. 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 1:10,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Mark3 antibody [EPR633Y] (ab52626)

All lanes : Anti-Mark3 antibody [EPR633Y] (ab52626) at 1/2000 dilution (purified)

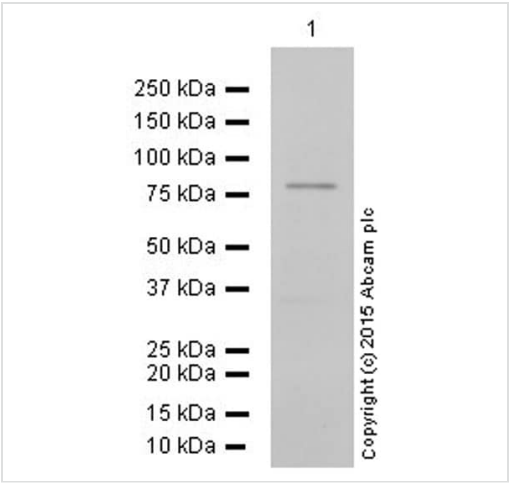
Lane 1 : K562 whole cell lysate
Lane 2 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Observed band size: 86 kDa

Blocking and dilution buffer: 5% NFDM/TBST



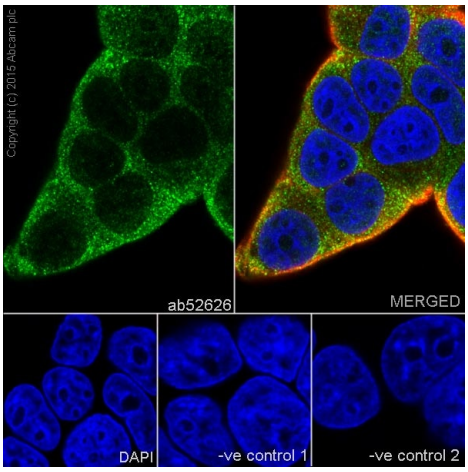
Western blot - Anti-Mark3 antibody [EPR633Y] (ab52626)

Anti-Mark3 antibody [EPR633Y] (ab52626) at 10 µg (purified) + NIH/3T3 whole cell lysate at 10 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Observed band size: 86 kDa

Blocking and dilution buffer: 5% NFDM/TBST

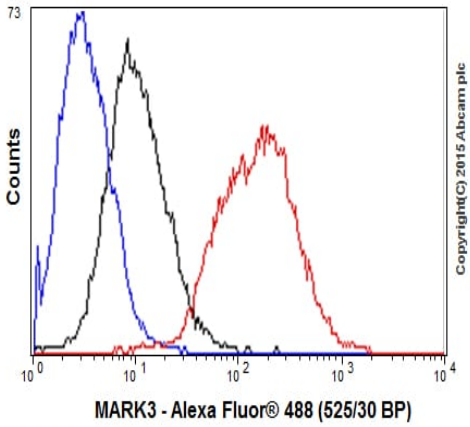


Immunocytochemistry/ Immunofluorescence - Anti-Mark3 antibody [EPR633Y] (ab52626)

Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling Mark3 with purified ab52626 at a dilution of 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

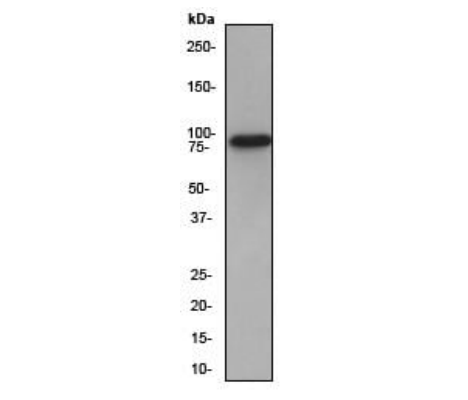
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



Flow Cytometry (Intracellular) - Anti-Mark3 antibody [EPR633Y] (ab52626)

Intracellular Flow Cytometry analysis ofHeLa cells labelling Mark3 with purified ab52626 at a dilution of 1/50 (red). Cells were fixed with 4% paraformaldehyde. An Alexa Fluorr® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



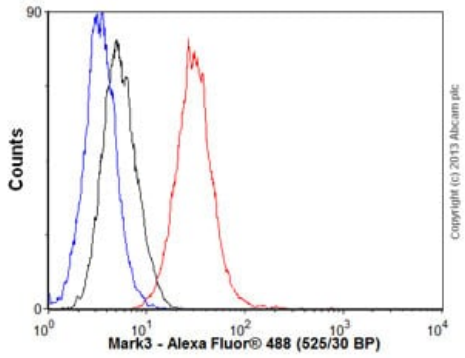
Western blot - Anti-Mark3 antibody [EPR633Y] (ab52626)

Anti-Mark3 antibody [EPR633Y] (ab52626) at 1/2000 dilution (unpurified) + HeLa cell lysate at 10 µg

Secondary

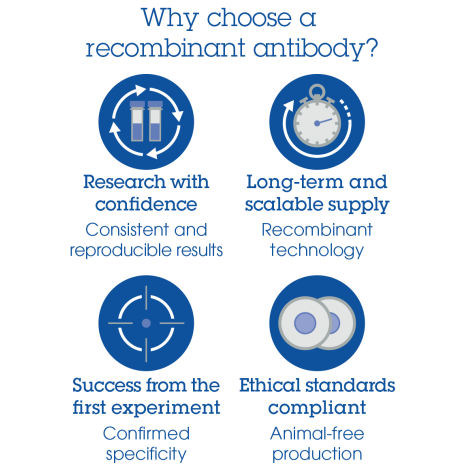
HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Observed band size: 86 kDa



Flow Cytometry (Intracellular) - Anti-Mark3 antibody [EPR633Y] (ab52626)

Overlay histogram showing HeLa cells stained with unpurified ab52626 (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 (unpurified ab52626, 1/1000 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/1x10^6 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.



Anti-Mark3 antibody [EPR633Y] (ab52626)

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