# abcam

## Product datasheet

## Anti-Mark3 antibody [EPR633Y] ab52626





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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

ClonalityMonoclonalClone numberEPR633Y

**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	*** <u>*</u>	1/20 - 1/60.
Flow Cyt (Intra)		1/30 - 1/1000.  ab172730 - Rabbit monoclonal lgG, 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.

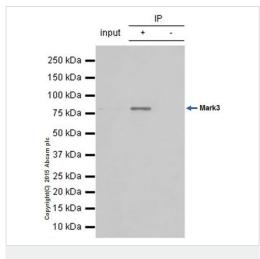
**Sequence similarities**Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. MARK subfamily.

Contains 1 KA1 (kinase-associated) domain.

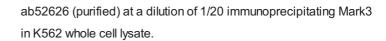
Contains 1 protein kinase domain.

Contains 1 UBA domain.

## Images



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



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

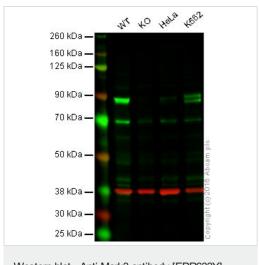
Lane 2 (+): ab52626 + K562 whole cell lysate.

Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab52626 in K562 whole cell lysate.

For western blotting, <u>ab131366</u> 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)

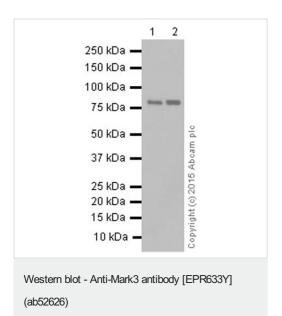
Lane 2: MARK3 knockout HAP1 cell lysate (40 µg)

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 <a href="mailto:ab8245">ab8245</a> (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 lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1:10,000 dilution for 1 hour at room temperature before imaging.



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

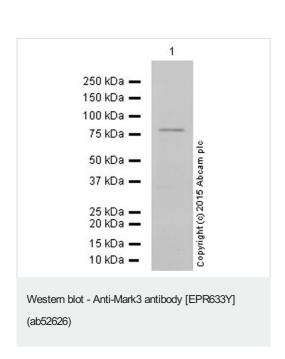
Lane 1 : K562 whole cell lysate
Lane 2 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

### **Secondary**

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

Observed band size: 86 kDa



Blocking and dilution buffer: 5% NFDM/TBST

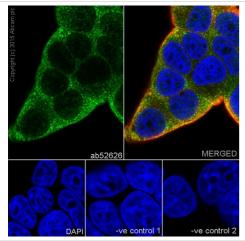
Anti-Mark3 antibody [EPR633Y] (ab52626) at 10  $\mu$ g (purified) + NIH/3T3 whole cell lysate at 10  $\mu$ g

#### **Secondary**

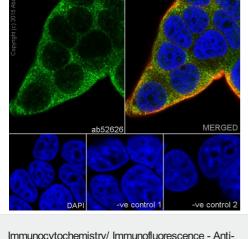
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 86 kDa

Blocking and dilution buffer: 5% NFDM/TBST



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



Intracellular Flow Cytometry analysis of HeLa 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 lgG (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.

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an

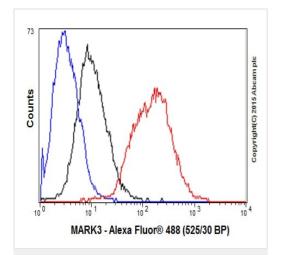
Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling Mark3 with purified ab52626 at a dilution of 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-

rabbit lgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse antitubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated

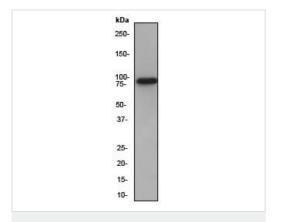
Control 1: primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG

Alexa Fluor® 488-conjugated goat anti-rabbit lgG (1/1000).

goat anti-mouse IgG (1/1000) were also used.



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



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

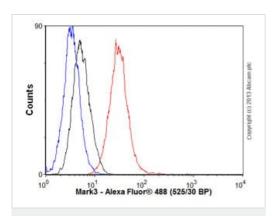
Anti-Mark3 antibody [EPR633Y] (ab52626) at 1/2000 dilution (unpurified) + HeLa cell lysate at 10 µg

## Secondary

(1/1000).

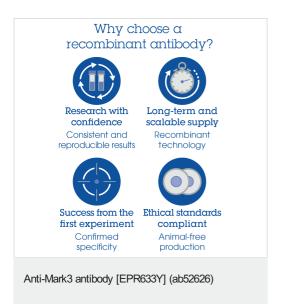
HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

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 Fluorr® 488 goat anti-rabbit lgG (H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1 $\mu$ g/1x10<sup>6</sup> 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.



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