abcam

Product datasheet

Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] ab32519

RabMAb

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Overview

Product name	Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336]
Description	Rabbit monoclonal [YE336] to Myosin Phosphatase + Myosin Phosphatase 2
Host species	Rabbit
Specificity	This antibody is specific for human Myosin Phosphatase 1 and Myosin Phosphatase 2.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, Jurkat, 293T, MCF-7, NIH/3T3 and C6 whole cell lysate. ICC/IF: HEK293 and NIH/3T3 cells Flow Cyt (intra): HEK293 and HeLa cells,
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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), 0.21% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	YE336
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32519 in the following tested applications.

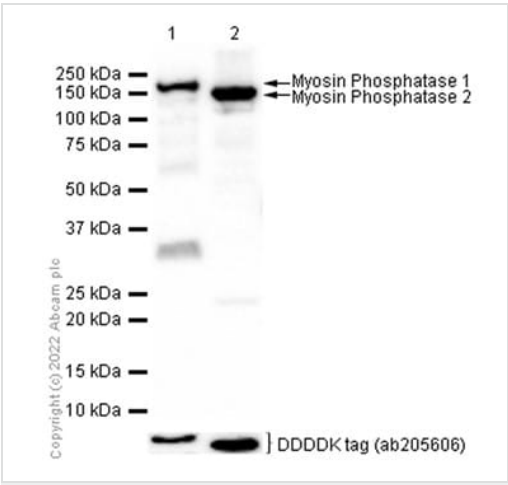
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/40 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (1)	1/1000 - 1/2000. Predicted molecular weight: 110 kDa. **For unpurified use at 1/5000.**
ICC/IF	★★★★☆ (1)	1/100.
IP		1/30. **For unpurified use at 1/80.**

Target

Cellular localization Myosin Phosphatase: Cytoplasm. Along actomyosin filaments and stress fibers. Myosin Phosphatase 2: Cytoplasmic

Images



Western blot - Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519)

All lanes : Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519) at 1/1000 dilution

Lane 1 : Human Myosin Phosphatase 1 full-length recombinant protein with DDDDK tag

Lane 2 : Human Myosin Phosphatase 2 full-length recombinant protein with DDDDK tag

Lysates/proteins at 0.01 µg per lane.

Secondary

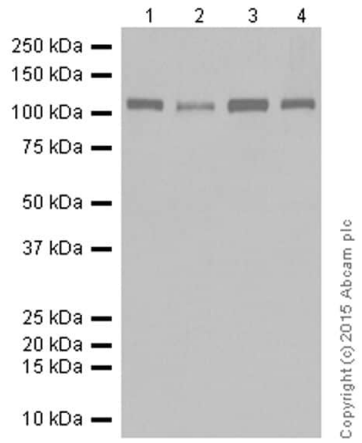
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 110 kDa

Observed band size: 150 kDa

Exposure time: 5 seconds

Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519)

All lanes : Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519) at 1/2000 dilution

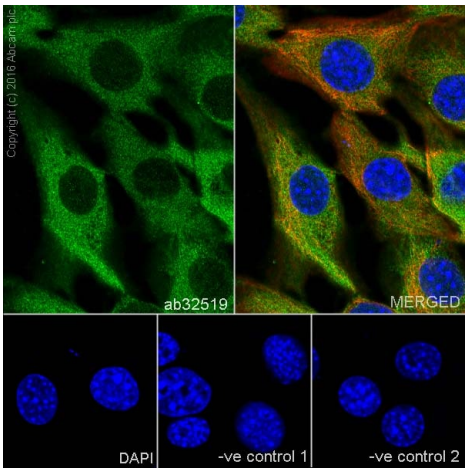
Lane 1 : HeLa whole cell lysate
Lane 2 : Jurkat whole cell lysate
Lane 3 : 293T whole cell lysate
Lane 4 : MCF-7 whole cell lysate

Lysates/proteins at 20 µg per lane.

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

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

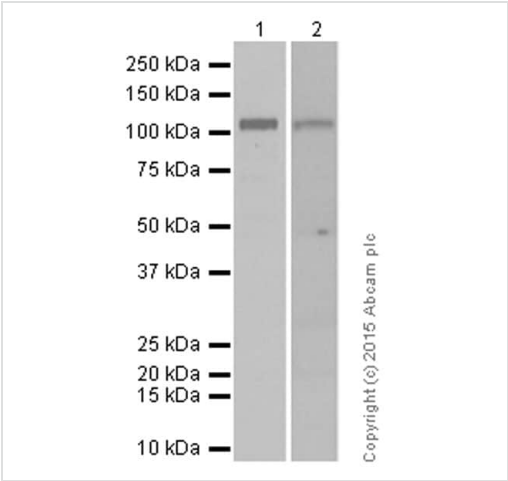
Blocking and Diluting buffer 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519)

Immunocytochemistry/Immunofluorescence analysis of NIH/3T3 cells labelling Myosin Phosphatase 1+Myosin Phosphatase 2 with purified ab32519 at 1/100. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% Triton X-100. **ab150077**, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counter-stained with **ab7291** anti-Tubulin (mouse mAb) followed by **ab150120**, AlexaFluor®594 goat anti-mouse secondary both at 1/1000. Nuclei were counterstained with DAPI (blue).

For negative control 1, rabbit primary antibody was used followed by anti-mouse secondary antibody (**ab150120**). For negative control 2, **ab7291** (mouse primary antibody) was used followed by anti-rabbit secondary antibody (**ab150077**).



Western blot - Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519)

All lanes : Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519) at 1/2000 dilution

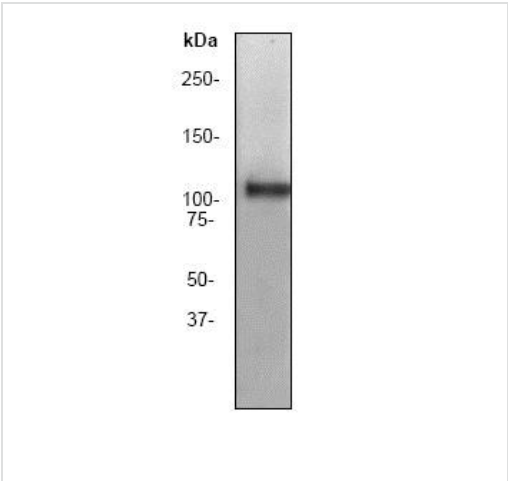
Lane 1 : NIH/3T3 whole cell lysate
Lane 2 : C6 whole cell lysate

Lysates/proteins at 20 µg per lane.

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

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

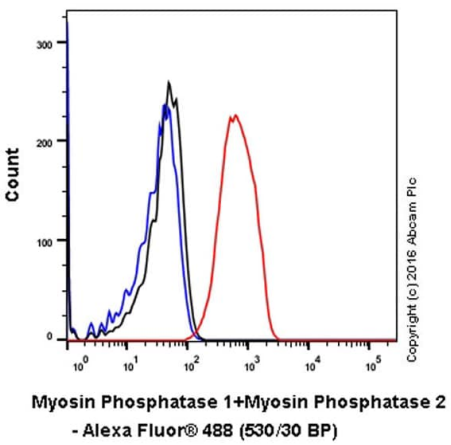
Blocking and Diluting buffer 5% NFDM/TBST



Western blot - Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519)

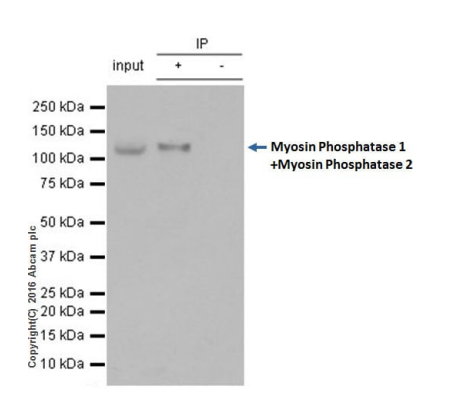
Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519) at 1/5000 dilution (unpurified) + 293T cell lysate.

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



Flow Cytometry (Intracellular) - Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519)

Intracellular Flow Cytometry analysis of HeLa cells labelling Myosin Phosphatase 1+Myosin Phosphatase 2 (red) with purified ab32519 at dilution of 1/40. The secondary antibody used was goat anti rabbit IgG (FITC) at 1/500. Cells were fixed with 4% paraformaldehyde. Isotype control antibody was Rabbit monoclonal IgG (black). The blue line shows cells without incubation with primary antibody and secondary antibody.



Immunoprecipitation - Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519)

ab32519 at 1/30 dilution immunoprecipitating Myosin Phosphatase 1+Myosin Phosphatase 2 in HeLa whole cell lysate observed at 110 KDa (lanes 1 and 2).

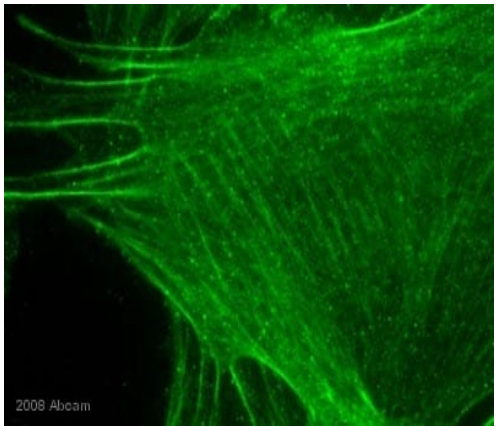
Lane 1 (input): HeLa whole cell lysate 10ug

Lane 2 (+): ab32519 + HeLa whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32519 in HeLa whole cell lysate

For western blotting, ab32519 was used followed by VeriBlot for IP Detection Reagent (HRP) (**ab131366**) for detection at a dilution of 1/10,000.

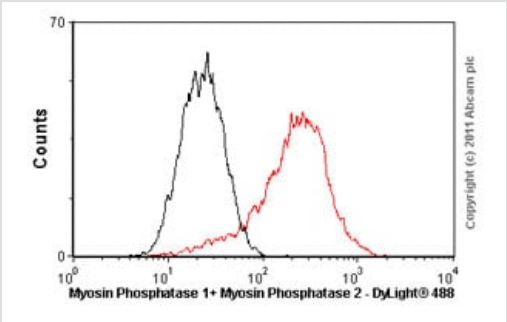
Blocking and Diluting buffer and concentration: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519)

This image is courtesy of an anonymous Abreview

ab32519 staining mouse fibroblast cells by ICC/IF. Cells were PFA fixed, permeabilized in Triton X-100 and blocked in 1% BSA for 30 minutes at 25°C. The primary antibody was diluted 1/200 and incubated with sample for 4 hours at 25°C. An Alexa Fluor® 488 conjugated goat monoclonal to rabbit, diluted 1/500 was used as the secondary.



Flow Cytometry (Intracellular) - Anti-Myosin Phosphatase + Myosin Phosphatase 2 antibody [YE336] (ab32519)

Overlay histogram showing HEK293 cells stained with unpurified ab32519 (red line). The cells were fixed with 4% paraformaldehyde (10 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 (ab32519, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 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. Acquisition of >5,000 events was performed. This antibody gave a positive signal in

HEK293 cells fixed with 100% methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

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