abcam

Product datasheet

Anti-Moesin antibody [EP1863Y] ab52490

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Moesin antibody [EP1863Y]
Description	Rabbit monoclonal [EP1863Y] to Moesin
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Moesin aa 450-550 (C terminal). The exact sequence is proprietary. Database link: **P26038** (Peptide available as **ab201545**)
Positive control	WB: Hela, Wild-type HAP1, HeLa and Raji whole cell lysate IHC-P: Human tonsil tissue ICC/IF: HeLa 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**.

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: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EP1863Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52490 in the following tested applications.

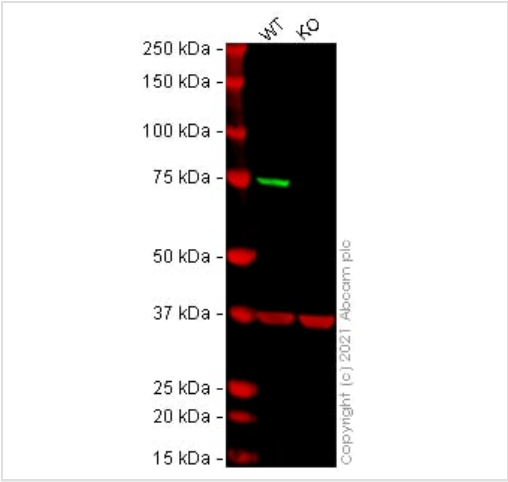
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/30 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ **(2)**	1/100 - 1/250.
WB	★★★★★ **(3)**	1/20000. Detects a band of approximately 68 kDa (predicted molecular weight: 68 kDa). **For unpurified use at 1/1000 - 1/10000.**
IP		1/20 - 1/70.
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

Target

Function	Probably involved in connections of major cytoskeletal structures to the plasma membrane.
Tissue specificity	In all tissues and cultured cells studied.
Sequence similarities	Contains 1 FERM domain.
Post-translational modifications	Phosphorylation on Thr-558 is crucial for the formation of microvilli-like structures.
Cellular localization	Cell membrane. Cytoplasm > cytoskeleton. Apical cell membrane. Cell projection > microvillus membrane. Phosphorylated form is enriched in microvilli-like structures at apical membrane (By similarity). Increased cell membrane localization of both phosphorylated and non-phosphorylated forms seen after thrombin treatment.

Images



Western blot - Anti-Moesin antibody [EP1863Y] (ab52490)

All lanes : Anti-Moesin antibody [EP1863Y] (ab52490) at 1/1000 dilution

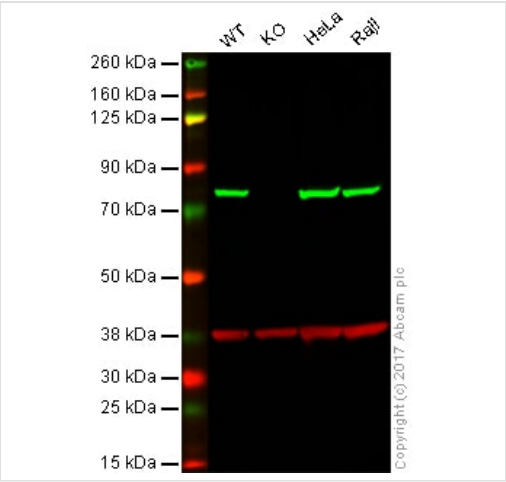
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MSN knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 75 kDa

False colour image of Western blot: Anti-Moesin antibody [EP1863Y] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab52490 was shown to bind specifically to Moesin. A band was observed at 75 kDa in wild-type HeLa cell lysates with no signal observed at this size in MSN knockout cell line **ab265020** (knockout cell lysate **ab257542**). To generate this image, wild-type and MSN knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-Moesin antibody [EP1863Y] (ab52490)

All lanes : Anti-Moesin antibody [EP1863Y] (ab52490) at 1/1000 dilution

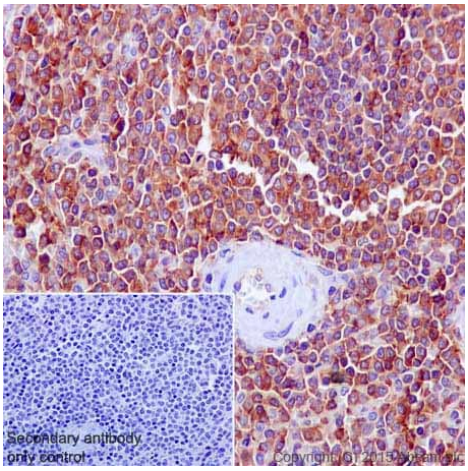
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Moesin knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : Raji whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 68 kDa

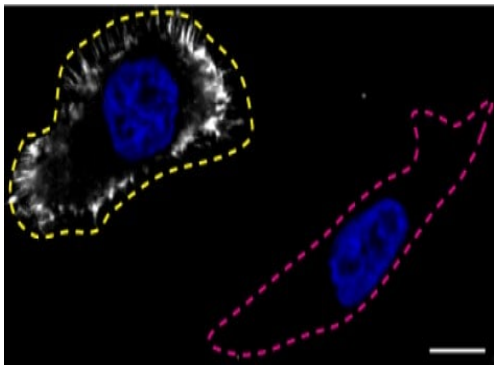
Lanes 1 - 4: Merged signal (red and green). Green - ab52490 observed at 75 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab52490 was shown to specifically react with Moesin in wild-type HAP1 cells as signal was lost in Moesin knockout cells. Wild-type and Moesin knockout samples were subjected to SDS-PAGE. Ab52490 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® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



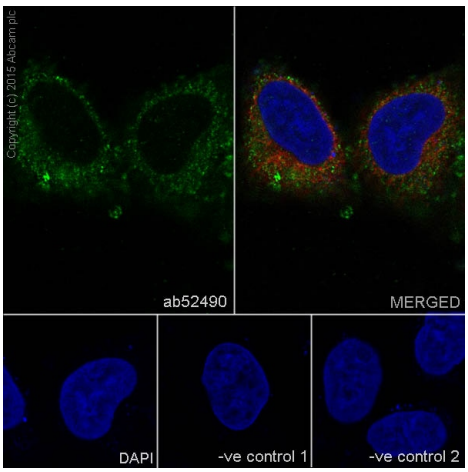
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Moesin antibody [EP1863Y] (ab52490)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human spleen tissue labelling Moesin with purified ab52490 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a goat anti-rabbit IgG H&L (HRP) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-Moesin antibody [EP1863Y] (ab52490)

ab52490 was shown to react with MSN in wild-type HeLa cells in Immunocytochemistry with loss of signal observed in MSN knockout cell line **ab265020**. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1/10000. The cells were then incubated with ab52490 at 1/200 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880). These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

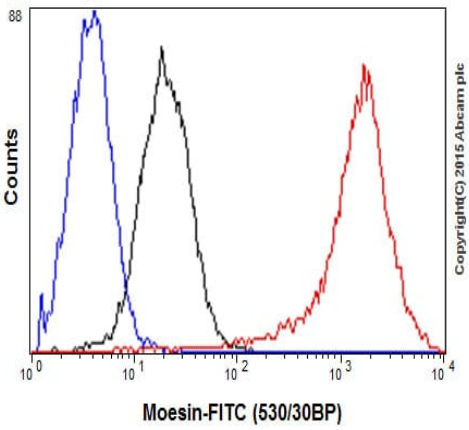


Immunocytochemistry/ Immunofluorescence - Anti-Moesin antibody [EP1863Y] (ab52490)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) cells labelling Moesin with purified ab52490 at 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 (1/500) 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.

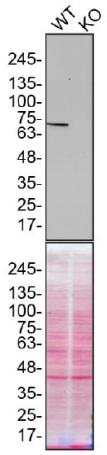
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



Flow Cytometry (Intracellular) - Anti-Moesin antibody [EP1863Y] (ab52490)

Intracellular Flow Cytometry analysis of HeLa cells labelling Moesin with purified ab52490 at 1/30 (red). Cells were fixed with 4% paraformaldehyde. A FITC-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-Moesin antibody [EP1863Y] (ab52490)

All lanes : Anti-Moesin antibody [EP1863Y] (ab52490) at 1/1000 dilution

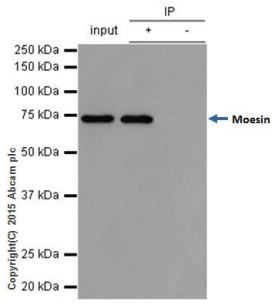
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MSN knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 68 kDa

ab52490 was shown to react with MSN in wild-type HeLa cells in Western blot with loss of signal observed in MSN knockout cell line **ab265020** (MSN knockout cell lysate **ab257542**). Wild-type HeLa and MSN knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab52490 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at 0.2ug/mL before imaging. These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

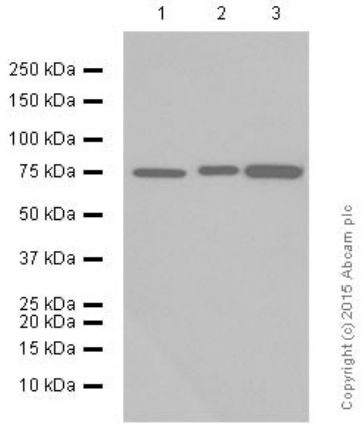


Immunoprecipitation - Anti-Moesin antibody [EP1863Y] (ab52490)

ab52490 (purified) at 1/20 immunoprecipitating Moesin in HeLa whole cell lysate. 10 ug of cell lysate was present in the input. For western blotting, a HRP-conjugated Veriblot for IP Detection Reagent (**ab131366**) (1/10,000) was used for detection. A rabbit monoclonal IgG (**ab172730**) was used intead of **ab128913** as a negative control (Lane 3).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Moesin antibody [EP1863Y] (ab52490)

All lanes : Anti-Moesin antibody [EP1863Y] (ab52490) at 1/20000 dilution (purified)

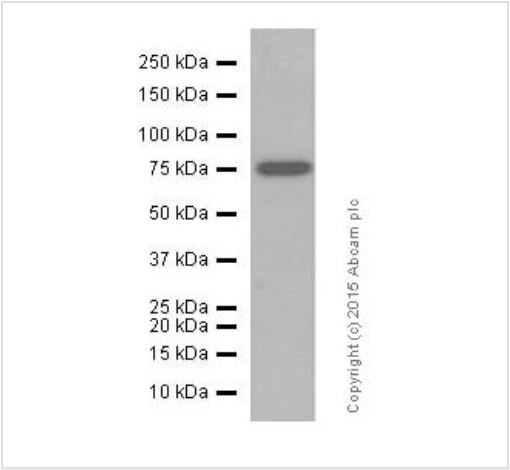
Lane 1 : HeLa cell lysate
Lane 2 : Raji cell lysate
Lane 3 : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 68 kDa

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



Western blot - Anti-Moesin antibody [EP1863Y] (ab52490)

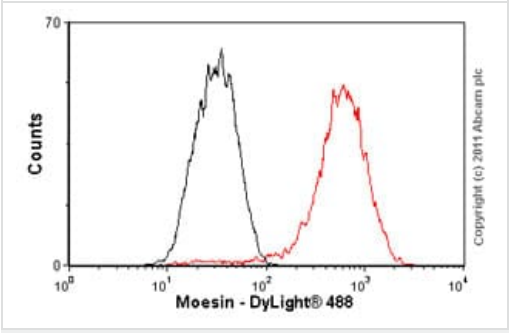
Anti-Moesin antibody [EP1863Y] (ab52490) at 1/50000 dilution (purified) + C6 cell lysate at 20 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

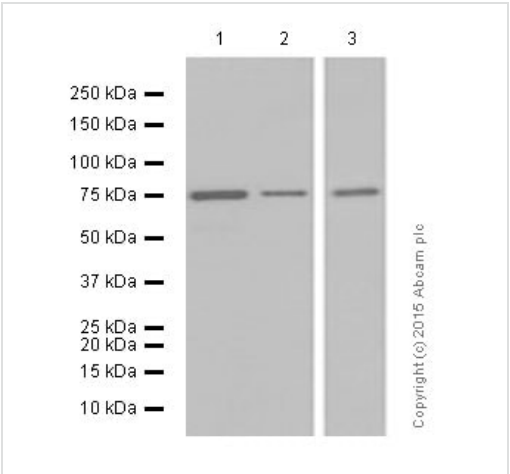
Predicted band size: 68 kDa

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



Flow Cytometry (Intracellular) - Anti-Moesin antibody [EP1863Y] (ab52490)

Overlay histogram showing HeLa cells stained with unpurified ab52490 (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 (ab52490, 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.



Western blot - Anti-Moesin antibody [EP1863Y] (ab52490)

All lanes : Anti-Moesin antibody [EP1863Y] (ab52490) at 1/20000 dilution (purified)

Lane 1 : Neuro-2a cell lysate
Lane 2 : Mouse heart lysate
Lane 3 : Rat heart lysate

Lysates/proteins at 20 µg per lane.

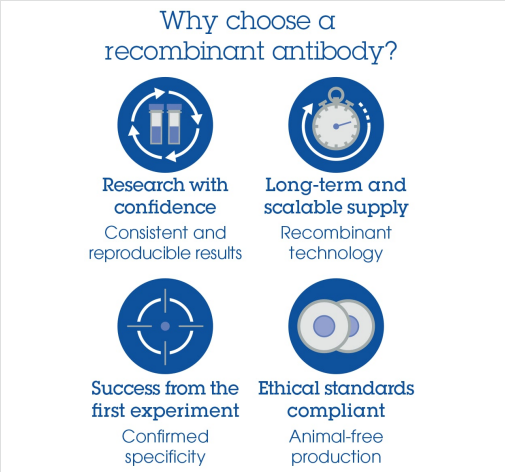
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 68 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Anti-Moesin antibody [EP1863Y] (ab52490)

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