abcam

Product datasheet

Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free ab283513

Recombinant RabMAb

12 Images

Overview

Product name Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free

Description Rabbit monoclonal [EPR24753-28] to Cofilin (phospho S3) - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: WB, Dot blot, IHC-P, ICC/IF, Flow Cyt (Intra)

Unsuitable for: 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, 293T, NIH/3T3 and C6 lysates all without alkaline phosphatase treatment. IHC-P:

> Human breast carcinoma, Human stomach, Mouse colon, Rat colon tissues all without alkaline phosphatase treatment. ICC/IF: HeLa cells without alkaline phosphatase treatment. Flow Cyt-intra:

HeLa cell. Dot Blot: Cofilin-1 (phospho S3) peptide.

General notes ab283513 is the carrier-free version of ab283500.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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.

Storage buffer pH: 7.20

Constituent: 100% PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR24753-28

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab283513 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		Use at an assay dependent concentration. Predicted molecular weight: 18 kDa.
Dot blot		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

Function Controls reversibly actin polymerization and depolymerization in a pH-sensitive manner. It has the

ability to bind G- and F-actin in a 1:1 ratio of cofilin to actin. It is the major component of

intranuclear and cytoplasmic actin rods.

Tissue specificity Widely distributed in various tissues.

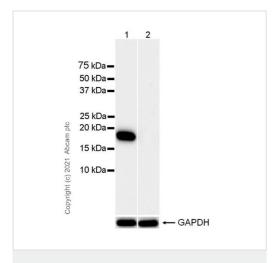
Sequence similaritiesBelongs to the actin-binding proteins ADF family.

Contains 1 ADF-H domain.

Post-translational Phosphorylated on Ser-3 in resting cells.

Nucleus matrix. Cytoplasm > cytoskeleton. Almost completely in nucleus in cells exposed to heat shock or 10% dimethyl sulfoxide.

Images



Western blot - Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free (ab283513)

All lanes : Anti-Cofilin (phospho S3) antibody [EPR24753-28] (ab283500) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate (Untreated membrane)

Lane 2 : HeLa(human cervix adenocarcinoma epithelial cell), whole cell lysate (Phosphatase treated membrane)

Lysates/proteins at 10 µg per lane.

Secondary

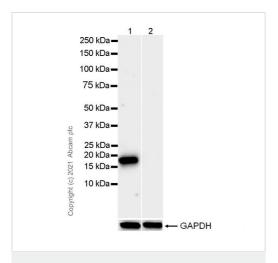
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 18 kDa **Observed band size:** 18 kDa

This data was developed using <u>ab283500</u>, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 37 seconds



Western blot - Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free (ab283513)

All lanes : Anti-Cofilin (phospho S3) antibody [EPR24753-28] (ab283500) at 1/1000 dilution

Lane 1: 293T (human embryonic kidney epithelial cell), whole cell lysate (Untreated membrane)

Lane 2: 293T (Phosphatase treated membrane)

Lysates/proteins at 10 µg per lane.

Secondary

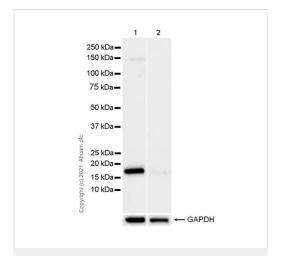
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Predicted band size: 18 kDa **Observed band size:** 18 kDa

This data was developed using <u>ab283500</u>, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 15 seconds



Western blot - Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free (ab283513)

All lanes : Anti-Cofilin (phospho S3) antibody [EPR24753-28] (ab283500) at 1/1000 dilution

Lane 1 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate (Untreated membrane)

Lane 2: NIH/3T3 (Phosphatase treated membrane)

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Predicted band size: 18 kDa **Observed band size:** 18 kDa

Exposure time: 3 seconds

This data was developed using ab283500, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST

All lanes: Anti-Cofilin (phospho S3) antibody [EPR24753-28] (ab283500) at 1/1000 dilution

Lane 1: C6 (rat glial tumor glial cell), whole cell lysate (Untreated membrane)

Lane 2: C6 (Phosphatase treated membrane)

Lysates/proteins at 10 µg per lane.

Secondary

Predicted band size: 18 kDa

All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Observed band size: 18 kDa

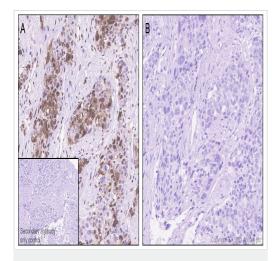
This data was developed using ab283500, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 10 seconds

250 kDa-150 kDa-100 kDa-75 kDa= 50 kDa-을 37 kDa 🗕 25 kDa = 20 kDa = ⊙ 15 kDa= 10 kDa-GAPDH Western blot - Anti-Cofilin (phospho S3) antibody

[EPR24753-28] - BSA and Azide free (ab283513)



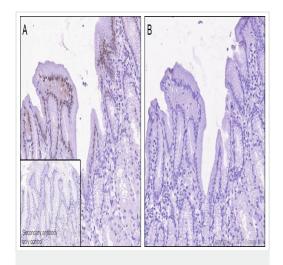
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free (ab283513)

This data was developed using <u>ab283500</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labelling Cofilin (phospho S3) with <u>ab283500</u> at 1/5000 (0.121 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Positive staining on human breast carcinoma without alkaline phosphatase treatment (image A). (PMID: 27799793) No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with <u>ab283500</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



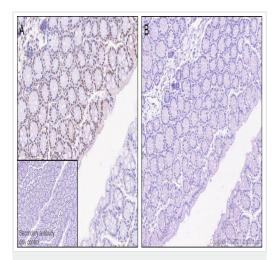
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free (ab283513)

This data was developed using <u>ab283500</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human stomach tissue labelling Cofilin (phospho S3) with <u>ab283500</u> at 1/5000 (0.121 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Positive staining on human stomach without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with <u>ab283500</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



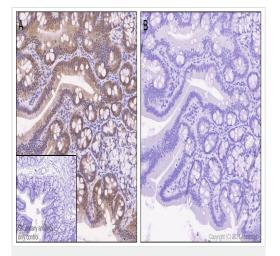
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free (ab283513)

This data was developed using <u>ab283500</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labelling Cofilin (phospho S3) with ab283500 at 1/5000 (0.121 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse colon without alkaline phosphatase treatment (image A). (PMID: 15649475) No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with ab283500 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



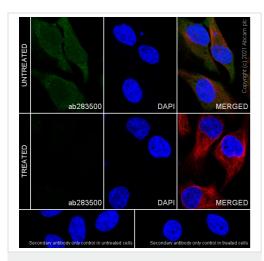
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free (ab283513)

This data was developed using <u>ab283500</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Rat colon tissue labelling Cofilin (phospho S3) with <u>ab283500</u> at 1/5000 (0.121 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on rat colon without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with <u>ab283500</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

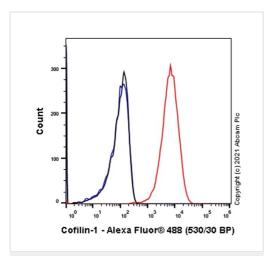


Immunocytochemistry/ Immunofluorescence - Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free (ab283513)

This data was developed using <u>ab283500</u>, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling Cofilin (phospho S3) with ab283500 at 1/1000 (0.604 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing nuclear and cytoplasmic staining in HeLa cells, no staining in HeLa treated with Alkaline Phosphatase (37°C) for 1 hour is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

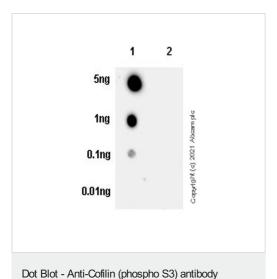
Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.



Flow Cytometry (Intracellular) - Anti-Cofilin (phospho S3) antibody [EPR24753-28] - BSA and Azide free (ab283513)

This data was developed using <u>ab283500</u>, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling Cofilin (phospho S3) with ab283500 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



[EPR24753-28] - BSA and Azide free (ab283513)

This data was developed using <u>ab283500</u>, the same antibody clone in a different buffer formulation.

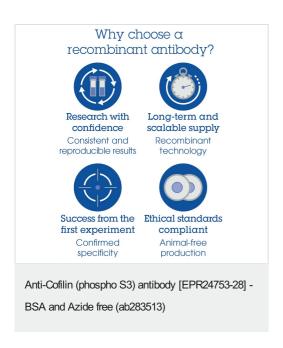
Dot blot analysis of Cofilin (phospho S3) using <u>ab283500</u> at 1/1000 (0.604 ug/ml) followed by a Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (<u>ab97051</u>) at 1/100,000 dilution.

Lane 1: Cofilin-1 (phospho S3) peptide

Lane 2: Cofilin-1 non-phospho peptide

Exposure time: 3 minutes

Blocking and diluting buffer and concentration: 5% NFDM/TBST



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