abcam

ab12866

Anti-Cofilin (phospho S3) antibody

Lab Essentials

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.3 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.1% BSA
Form	Liquid
Clonality	Polyclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Purification technique	Affinity purification Immunogen
Concentration	0.7 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	1/250
Notes	-

Expected

Species	African green monkey
Dilution info	-
Notes	-

Species	Mouse
Dilution info	-
Notes	-

Species	Dog
Dilution info	-
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	3-5 µg for 10^5 Cells
Notes	-

Expected

Species	African green monkey
Dilution info	-
Notes	-

Species	Mouse
Dilution info	-
Notes	-

Species	Dog
Dilution info	-

Notes	-

Species	Rat
Dilution info	3-5 µg for 10^6 Cells
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	African green monkey
Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Dog
Dilution info	1/1000
Notes	-

Expected

Species	Rat
Dilution info	1/1000
Notes	-

Target data

See full target information CFL1 phospho S3

Function	Binds to F-actin and exhibits pH-sensitive F-actin depolymerizing activity (PubMed:11812157). In conjunction with the subcortical maternal complex (SCMC), plays an essential role for zygotes to progress beyond the first embryonic cell divisions via regulation of actin dynamics (PubMed:15580268). Required for the centralization of the mitotic spindle and symmetric division of zygotes (By similarity). Plays a role in the regulation of cell morphology and cytoskeletal organization in epithelial cells (PubMed:21834987). Required for the up-regulation of atypical chemokine receptor ACKR2 from endosomal compartment to cell membrane, increasing its efficiency in chemokine uptake and degradation (PubMed:23633677). Required for neural tube morphogenesis and neural crest cell migration (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Cofilin-1 also known just as cofilin is a small actin-modulating protein with a molecular weight of approximately 19 kDa. It is part of the actin chemical structure regulation and plays an important role in actin filament dynamics by severing and depolymerizing actin filaments. Cofilin-1 expresses widely in both muscle and non-muscle tissues modulating the actin cytoskeleton which is critical for cell movement shape and various signaling pathways. The phosphorylated form of this protein known as phospho-cofilin or p-cofilin is another significant state to consider.
Biological function summary	Through its interaction with actin filaments cofilin-1 functions to regulate the length and turnover of the actin cytoskeleton within cells. Cofilin-1 is part of a critical complex where it binds to actin monomers and filaments to facilitate the disassembly and recycling of actin. This regulation impacts cellular activities such as migration endocytosis and division influencing how cells respond to internal and external signals. Cofilin-1's ability to bind and sever actin filaments forms an important part of its functional mechanism altering the cytoskeletal architecture for various cellular processes.
Pathways	Cofilin-1 participates principally in the actin dynamics component of the cytoskeleton remodeling pathway. Within this framework cofilin-1 acts alongside

	other proteins like actin-depolymerizing factor (ADF) and tropomyosin influencing assembly and disassembly cycles of actin filaments. The Rho family of GTPases regulates cofilin-1 activity through signaling pathways such as the Rho/ROCK/LIMK pathway. This signaling regulation affects actin filament organization impacting cellular processes like axonal guidance and directed cell movement.
Associated diseases and disorders	There is a strong connection between cofilin-1 dysregulation and neurodegenerative diseases such as Alzheimer's disease and certain types of cancer. In Alzheimer's disease cofilin-1 activity influenced by abnormal cofilin phosphorylation forms part of the pathological mechanisms that lead to synaptic dysfunction and neurofibrillary tangles. In the context of cancer cofilin-1 collaborates with proteins such as RhoA and LIM kinase contributing to cancer cell invasion and metastasis by reorganizing the actin cytoskeleton to facilitate cell motility. Understanding these interactions helps in targeting therapeutic strategies against these conditions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

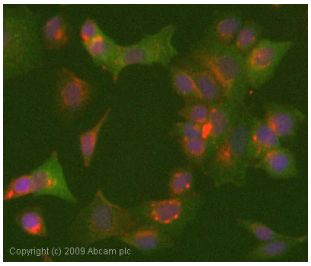
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

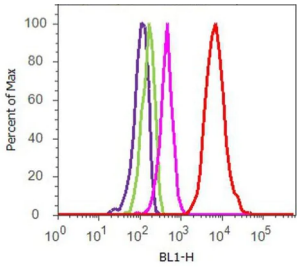
7 product images



Immunocytochemistry/ Immunofluorescence - Anti-Cofilin (phospho S3) antibody (ab12866)

Cofilin (phospho S3) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cofilin (phospho S3) antibody

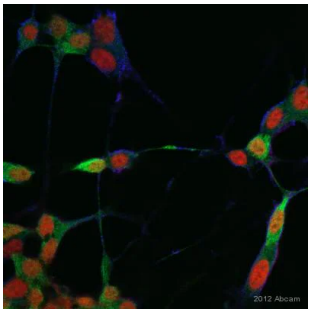
ICC/IF image of ab12866 stained MCF7 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab12866, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Flow Cytometry - Anti-Cofilin (phospho S3) antibody (ab12866)

Cofilin (phospho S3) Flow Cytometry staining using rabbit Anti-Cofilin (phospho S3) antibody

Flow Cytometry analysis of U-87 MG cells labeling Cofilin (phospho S3) with ab12866. Cells were fixed with 70% ethanol for 10 minutes, permeabilized with 0.25% Triton™ X-100 for 20 minutes, and blocked with 5% BSA for 30 minutes at room temperature. Cells were labeled with Anti-Cofilin (phospho S3) antibody (ab12866, red) or with rabbit isotype control (pink) at 3-5 ug/million cells in 2.5% BSA. After incubation at room temperature for 2 hours, the cells were labeled with Alexa Fluor® 488 Goat Anti-Rabbit Secondary Antibody at a dilution of 1/400 for 30 minutes at room temperature. The representative 10,000 cells were acquired and analyzed for each sample. The purple histogram represents unstained control cells and the green histogram represents no-primary-antibody control.

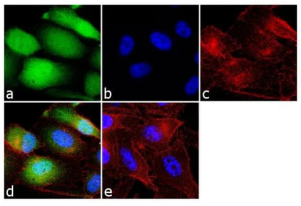


This image is courtesy of an anonymous customer review.

Immunocytochemistry/ Immunofluorescence - Anti-Cofilin (phospho S3) antibody (ab12866)

Cofilin (phospho S3) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cofilin (phospho S3) antibody

Immunocytochemistry/ Immunofluorescence analysis of human neuroblastoma cells labeling Cofilin (phospho S3) with ab12866 at 1/500 dilution. Cells were fixed in paraformaldehyde, permeabilized for 15 minutes in 0.01% Triton X-100, blocked using 5% serum for 30 minutes at 20°C, then incubated with ab12866 at a 1/500 dilution for 2 hours at 20°C. The secondary used was a Dylight 488 conjugated donkey anti-rabbit IgG (H+L) used at a 1/500 dilution.

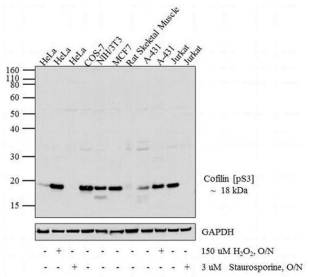


Immunocytochemistry/ Immunofluorescence - Anti-Cofilin (phospho S3) antibody (ab12866)

Cofilin (phospho S3) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Cofilin (phospho S3) antibody

Immunofluorescence analysis of Phospho-Cofilin pSer3 was done on 70% confluent log phase PC-3 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with ab12866 at 1:250 dilution in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate at a dilution of 1:2000 for 45 minutes at room temperature (Panel a:

green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI. F-actin (Panel c: red) was stained with Rhodamine Phalloidin (1:300). Panel d is a merged image showing cytoplasmic and nuclear localization. Panel e is a no primary antibody control. The images were captured at 60X magnification.



Western blot - Anti-Cofilin (phospho S3) antibody (ab12866)

All lanes:
Western blot - Anti-Cofilin (phospho S3) antibody (ab12866) at 1 µg/mL

Lane 1:
HeLa whole cell extract at 20 µg

Lane 2:
HeLa treated for overnight with 150 uM of H2O2 whole cell extract at 20 µg

Lane 3:
HeLa treated for overnight with 3 uM of Staurosporine whole cell extract at 20 µg

Lane 4:
COS-7 whole cell extract at 20 µg

Lane 5:
NIH/3T3 (Mouse embryo fibroblast cell line) whole cell extract at 20 µg

Lane 6:
MCF7 whole cell extract at 20 µg

Lane 7:
Rat Skeletal Muscle whole cell extract at 20 µg

Lane 8:
A-431 whole cell extract at 20 µg

Lane 9:
A-431 treated for overnight with 150 uM of H2O2 whole cell extract at 20 µg

Lane 10:
Jurkat whole cell extract at 20 µg

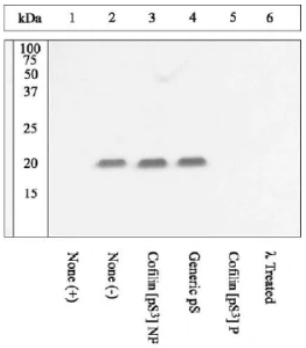
Lane 11:
Jurkat treated for overnight with 3 uM of Staurosporine whole cell extract at 20 µg

Secondary

All lanes:
Goat anti-rabbit IgG (H+L), HRP conjugate at 1/2500 dilution

Predicted band size: 18 kDa

Observed band size: 18 kDa



Western blot - Anti-Cofilin (phospho S3) antibody (ab12866)

Cofilin (phospho S3) Western blot staining using rabbit Anti-Cofilin (phospho S3) antibody

Peptide Competition and Phosphatase Treatment
Lysates prepared from MDCK cells treated with staurosporine (1) or left untreated (2-6) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were either left untreated (1-5) or treated with lambda phosphatase (6), blocked with a 5% BSA-TBST buffer for one hour at room temperature, and incubated with the ab12866 antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2, 6), the non phosphopeptide corresponding to the immunogen (3), a generic phosphoserine-containing peptide (4) or, the phosphopeptide immunogen (5). After washing, membranes were incubated with goat F(ab)2 anti-rabbit IgG HRP conjugate and bands were detected using the Pierce SuperSignal method. The data show that only the peptide corresponding to cofilin [pS3] blocks the antibody signal. The data also show that phosphatase stripping eliminates the signal, verifying that the anti
All lanes:
Western blot - Anti-Cofilin (phospho S3) antibody (ab12866)

Predicted band size: 18 kDa

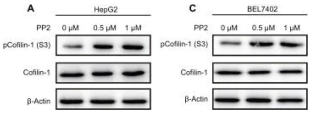


Image collected and cropped by CiteAb under a CC-BY license from the publication

Western blot - Anti-Cofilin (phospho S3) antibody (ab12866)

Cofilin (phospho S3) Western blot staining using rabbit Anti-Cofilin (phospho S3) antibody

Cofilin (phospho S3) western blot using anti-Cofilin (phospho S3) antibody ab12866. Publication image and figure legend from Pang, D., Yang, C., et al., 2020, Biol Open, PubMed 31988091.

ab12866 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab12866 please see the product overview.

Suppressed cofilin activity was implicated in PP2-inhibited cellular motility of HepG2 and BEL7402 cells. (A–D) Western blots and quantifications show increased phosphorylation of cofilin-1 in the cells treated with 0, 0.5 and 1.0 μM PP2 for 24 h. (E,F) Wound healing assay and quantifications show stably expressed mutant cofilin-1 (S3A) improves cellular motility of HepG2 cells under the treatment of PP2 (1 μM). (G–J) Western blots and quantifications show decreased protein levels of SSH-1 in the cells treated with 0, 0.5 and 1.0 μM PP2 for 24 h. **p<0.01, ***p<0.001. Scale bar: 200 μm.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.