abcam

Product datasheet

Anti-Cofilin (phospho S3) antibody ab12866

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概述

产品名称	Anti-Cofilin (phospho S3)**抗体**
描述	**兔多克隆抗体**to Cofilin (phospho S3)
宿主	Rabbit
经测试应用	**适用于:** WB, ICC/IF, Flow Cyt
种属反应性	**与反应:** Mouse, Rat, Dog, Human, African green monkey
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
常规说明	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

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.3 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol, 0.1% BSA PBS (without Mg2+ and Ca2+), BSA (IgG, protease free)
纯度	Immunogen affinity purified
克隆	**多克隆**
同种型	IgG

应用

The Abpromise guarantee	**Abpromise™承诺保证使用**ab12866**于以下的**经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	(6)	1/1000. Detects a band of approximately 20 kDa.
ICC/IF	(3)	1/250.
Flow Cyt		Use 3-5µg for 10^6 cells.

靶标

功能 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.

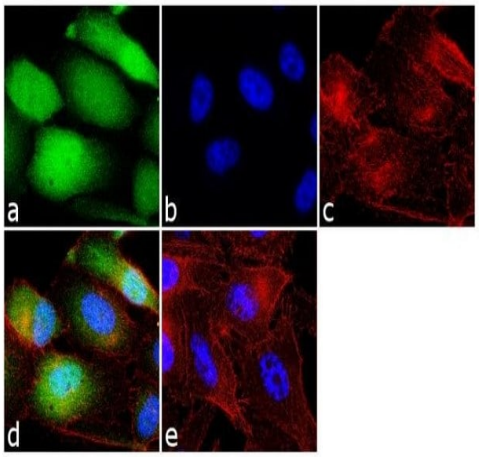
组织特异性 Widely distributed in various tissues.

序列相似性 Belongs to the actin-binding proteins ADF family.
Contains 1 ADF-H domain.

翻译后修饰 Phosphorylated on Ser-3 in resting cells.

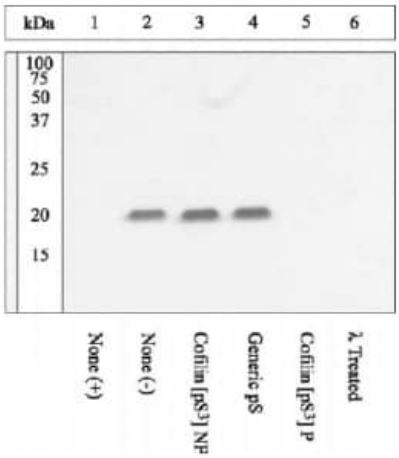
细胞定位 Nucleus matrix. Cytoplasm > cytoskeleton. Almost completely in nucleus in cells exposed to heat shock or 10% dimethyl sulfoxide.

图片



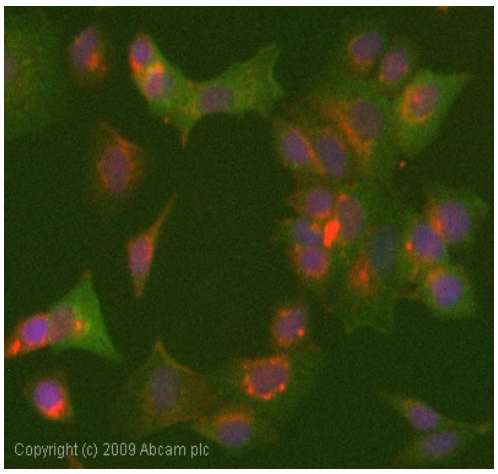
Immunocytochemistry/ Immunofluorescence - Anti-Cofilin (phospho S3) antibody (ab12866)

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.



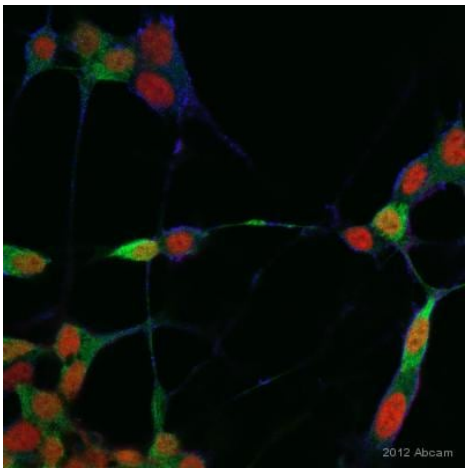
Western blot - Anti-Cofilin (phospho S3) antibody (ab12866)

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



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

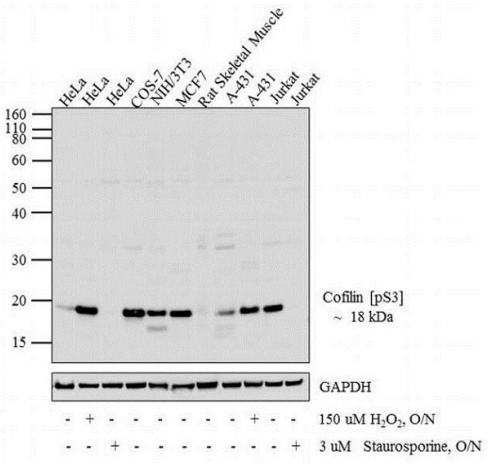
ICC/IF image of ab12866 stained MCF7 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab12866, 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.



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

This image is courtesy of an anonymous abreview.

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.



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

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

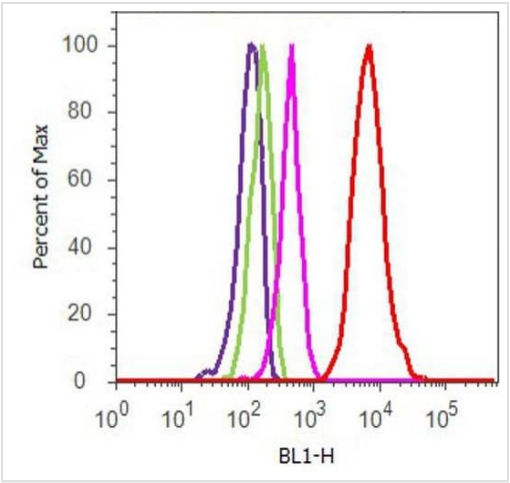
Lane 1 : HeLa whole cell extract
Lane 2 : HeLa treated for overnight with 150 uM of H2O2 whole cell extract
Lane 3 : HeLa treated for overnight with 3 uM of Staurosporine whole cell extract
Lane 4 : COS-7 whole cell extract
Lane 5 : NIH/3T3 (Mouse embryo fibroblast cell line) whole cell extract
Lane 6 : MCF7 whole cell extract
Lane 7 : Rat Skeletal Muscle whole cell extract
Lane 8 : A-431 whole cell extract
Lane 9 : A-431 treated for overnight with 150 uM of H2O2 whole cell extract
Lane 10 : Jurkat whole cell extract
Lane 11 : Jurkat treated for overnight with 3 uM of Staurosporine whole cell extract

Lysates/proteins at 20 µg per lane.

Secondary

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

Observed band size: 18 kDa



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

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.

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