abcam

Product datasheet

Anti-Actin antibody [ACTN05 (C4)] ab3280

33 Abreviews 444 References 7 Images

Overview

Product name	Anti-Actin antibody [ACTN05 (C4)]
Description	Mouse monoclonal [ACTN05 (C4)] to Actin
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, IP, WB, IHC-Fr, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Rabbit, Cow, Dog, Human, Pig, Dictyostelium discoideum, Physarum polycephalum **Predicted to work with:** Chicken
Immunogen	Chicken gizzard actin.
Positive control	HeLa cells.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.40 Preservative: 1.37% Imidazole Constituents: 5% Sodium molybdate, 5% Sodium orthovanadate, 1% Sodium fluoride, 65% Water
Purity	Protein G purified
Clonality	Monoclonal
Clone number	ACTN05 (C4)
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee	Our Abpromise guarantee covers the use of ab3280 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		Use at an assay dependent concentration. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IP	★★★★☆ (2)	Use at an assay dependent concentration. Use Protein G. This has been tested in IP on "denatured" cell lysate (treated with 5% SDS).
WB	★★★★★ (20)	Use at an assay dependent concentration. Detects a band of approximately 42 kDa (predicted molecular weight: 42 kDa).
IHC-Fr	★★★★★ (2)	1/250.
IHC-P	★★★★★ (2)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF	★★★★☆ (5)	Use at an assay dependent concentration. See Bryce et al. Fix cells with 4% paraformaldehyde and permeabilize with chilled methanol.

Target

Function

Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.

Involvement in disease

Defects in ACTA1 are the cause of nemaline myopathy type 3 (NEM3) [MIM:161800]. A form of nemaline myopathy. Nemaline myopathies are muscular disorders characterized by muscle weakness of varying severity and onset, and abnormal thread-or rod-like structures in muscle fibers on histologic examination. The phenotype at histological level is variable. Some patients present areas devoid of oxidative activity containg (cores) within myofibers. Core lesions are unstructured and poorly circumscribed.
Defects in ACTA1 are a cause of myopathy congenital with excess of thin myofilaments (MPCETM) [MIM:161800]. A congenital muscular disorder characterized at histological level by areas of sarcoplasm devoid of normal myofibrils and mitochondria, and replaced with dense masses of thin filaments. Central cores, rods, ragged red fibers, and necrosis are absent.
Defects in ACTA1 are a cause of congenital myopathy with fiber-type disproportion (CFTD) [MIM:255310]; also known as congenital fiber-type disproportion myopathy (CFTDM). CFTD is a genetically heterogeneous disorder in which there is relative hypotrophy of type 1 muscle fibers compared to type 2 fibers on skeletal muscle biopsy. However, these findings are not specific and can be found in many different myopathic and neuropathic conditions.

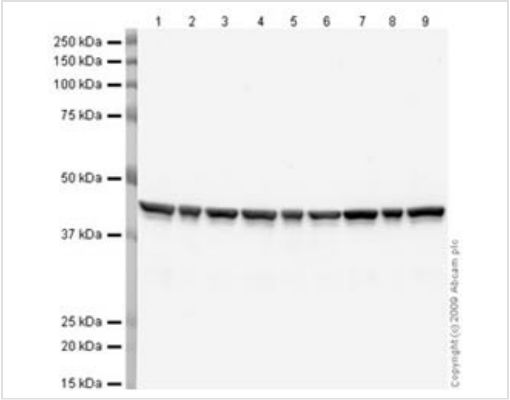
Sequence similarities

Belongs to the actin family.

Cellular localization

Cytoplasm > cytoskeleton.

Images



Western blot - Anti-Actin antibody [ACTN05 (C4)] (ab3280)

All lanes : Anti-Actin antibody [ACTN05 (C4)] (ab3280)

Lanes 1 & 4 & 7 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate
Lanes 2 & 5 & 8 : MDA-MB-231 (Human breast adenocarcinoma cell line) Whole Cell Lysate Whole Cell Lysate
Lanes 3 & 6 & 9 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Developed using the ECL technique.

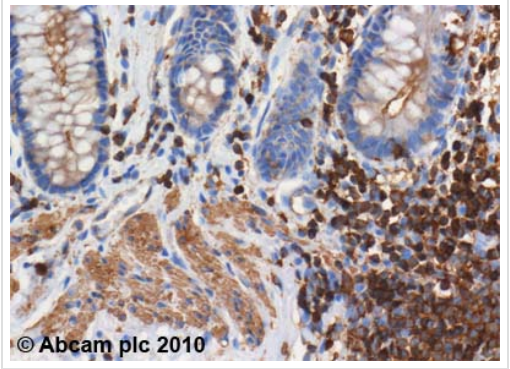
Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 42 kDa

Lanes: 1-3: 4^{O}C (x1 freeze/thaw)

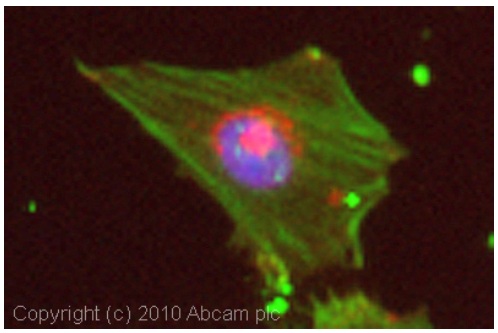
Lanes: 4-6: 4^{O}C (x2 freeze/thaws)

Lanes 7-9: 4^{O}C (x4 freeze/thaws)



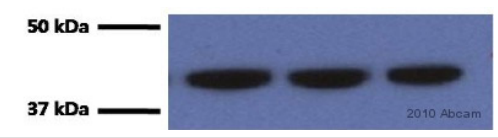
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Actin antibody [ACTN05 (C4)] (ab3280)

ab3280 (1µg/ml) staining actin in human colon using an automated system (DAKO Autostainer Plus). Using this protocol there is strong cytoplasmic staining throughout the tissue.
Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH6.1 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



Immunocytochemistry/ Immunofluorescence - Anti-Actin antibody [ACTN05 (C4)] (ab3280)

ICC/IF image of ab3280 stained Hepp cells. The cells were 4% formaldehyde 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 (ab3280, 10µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse 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.



Western blot - Anti-Actin antibody [ACTN05 (C4)] (ab3280)

This image is courtesy of an anonymous Abreview

All lanes : Anti-Actin antibody [ACTN05 (C4)] (ab3280) at 1/2000 dilution

Lane 1 : Mouse liver whole tissue lysate (mouse 1).
Lane 2 : Mouse liver whole tissue lysate (mouse 2).
Lane 3 : Mouse liver whole tissue lysate (mouse 3).

Lysates/proteins at 50 µg per lane.

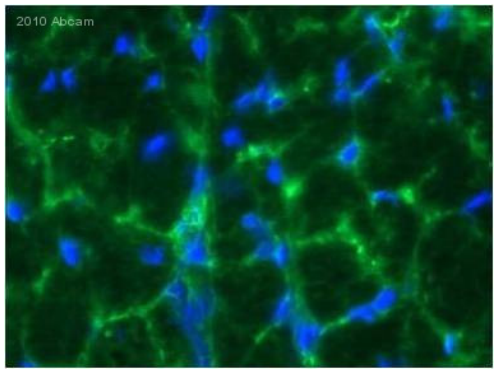
Secondary
All lanes : An HRP-conjugated goat anti-mouse IgG polyclonal. at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 42 kDa

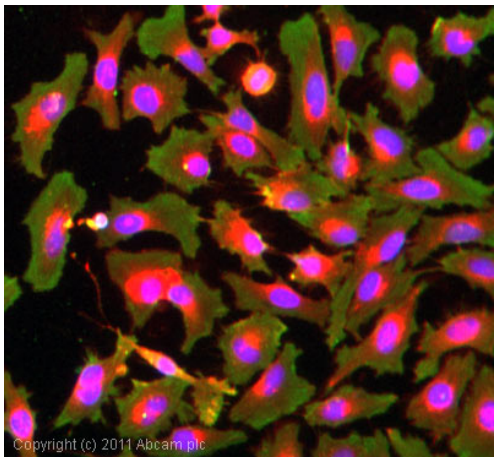
Blocking Step: 5% Milk for 30 minutes at 25°C.



Immunohistochemistry (Frozen sections) - Anti-Actin antibody [ACTN05 (C4)] (ab3280)

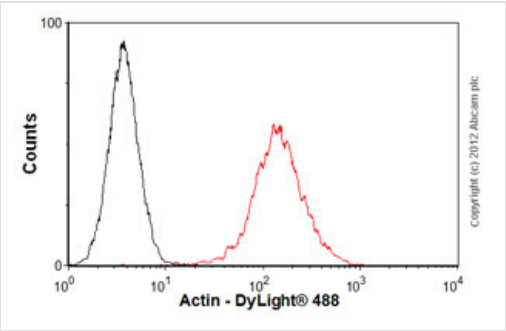
This image is courtesy of an anonymous Abreview

ab3280 staining Actin in mouse heart tissue sections by IHC-Fr (formaldehyde-fixed frozen sections). Tissue samples were fixed with formaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 5% serum for 1 hour at 25°C. The sample was incubated with primary antibody (1/250 in PBS-Tween) at 4°C for 12 hours. An Alexa Fluor®488-conjugated Goat polyclonal to mouse IgG (1/250) was used as secondary antibody. Nuclei were stained with DAPI.



Immunocytochemistry/ Immunofluorescence - Anti-Actin antibody [ACTN05 (C4)] (ab3280)

ICC/IF image of ab3280 stained HeLa cells. The cells were 100% methanol fixed (5 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 (ab3280, 5µg/ml) overnight at +4°C. The secondary antibody (green) was a goat anti-mouse DyLight® 488 (IgG - H&L, pre-adsorbed) (ab96879) used at a 1/250 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-Actin antibody [ACTN05 (C4)] (ab3280)

Overlay histogram showing HeLa cells stained with ab3280 (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 (ab3280, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was a goat anti-mouse DyLight® 488 (IgG; H+L) (ab96879) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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