abcam

Product datasheet

Anti-Actin antibody [C4] ab14128

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Overview

Product name	Anti-Actin antibody [C4]
Description	Mouse monoclonal [C4] to Actin
Host species	Mouse
Specificity	ab14128 recognizes all six isoforms of vertebrate actin, globular (G) and filamentous (F) forms; it is a pan-actin antibody that binds to an epitope in a highly conserved region of actin. ab14128 labels myotubes and stains myoblasts and fibroblasts, it does not interfere with actin polymerization to form filaments, at a ratio as high as one antibody per two actin monomers. In ELISA, ab14128 shows a strong reactivity with cytoplasmic actin and significant binding to gizzard, skeletal, arterial and cardiac actins, the antibody also shows significant binding to both Dictyostelium discoidum and Physarum polycephalum in ELISA. The clone number has been updated from (2Q1055) to (C4) both clone numbers name the same antibody clone.
Tested applications	**Suitable for:** ICC/IF, WB, ICC
Species reactivity	**Reacts with:** Chicken, Saccharomyces cerevisiae, Tetrahymena, Caenorhabditis elegans, Mammals, Dictyostelium discoideum, Physarum polycephalum, Vertebrata
Immunogen	Recombinant fragment corresponding to Chicken Actin aa 50-70 (N terminal). The immuogen is purified chicken gizzard actin. This is predominantly smooth muscle gamma-actin. Is a pan-actin which recognizes all actin isoforms. Sequence: KDSYVGDEAQ SKRGILTLKY Database link: P63270 Run BLAST with Run BLAST with
Epitope	The epitope recognized by the antibody appears to be located in the N-terminal two thirds of the actin molecule, possibly near amino acids 50-70.
Positive control	This antibody gave a positive signal in both Human Skeletal Muscle and Heart tissue lysates as well as the following whole cell lysates: A431; HEK293; MCF7; HeLa; MDA MB 231. ICC/IF: HeLa cells
General notes	For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Further dilutions can be made in assay buffer.

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: 7.40 Preservative: 0.05% Sodium azide Constituents: Tris glycine, 0.88% Sodium chloride
Purity	Ascites
Purification notes	Purified from ascites.
Clonality	Monoclonal
Clone number	C4
Isotype	IgG2b
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab14128** in the following tested applications.

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

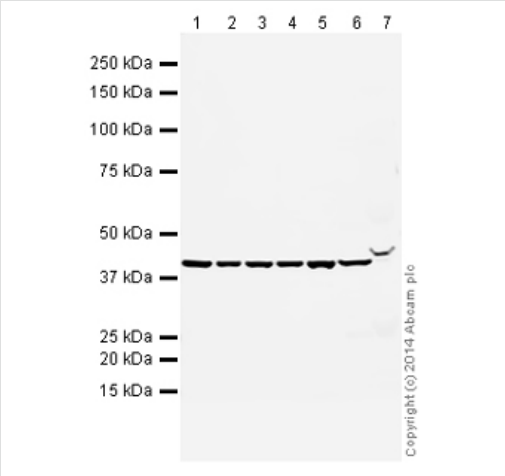
Application	Abreviews	Notes
ICC/IF		Use a concentration of 5 µg/ml.
WB	★★★★☆	Use a concentration of 1 - 20 µg/ml. Predicted molecular weight: 43 kDa. On muscle homogenates subject to SDS-PAGE, reacts relatively uniformly with a 43kDa protein present in skeletal, cardiac, gizzard and aorta tissues. Appears to react with all isoforms of actin found in these preparations and shows a strong reaction with the alpha actin found in skeletal, cardiac and arterial muscle.
ICC		1/500. Methanol fixed HeLa and NIH/3T3 cells

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 [C4] (ab14128)

All lanes : Anti-Actin antibody [C4] (ab14128) at 1/1000 dilution

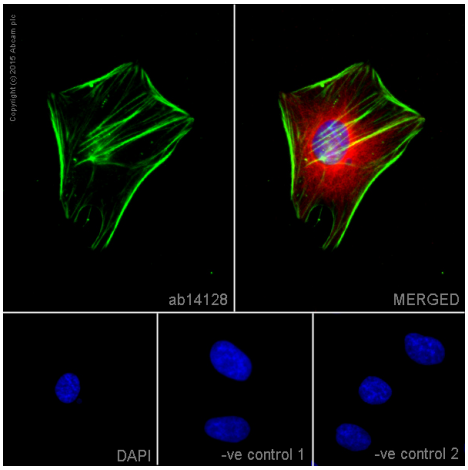
Lane 1 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate
Lane 3 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate
Lane 4 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 5 : MDA-MB-231 (Breast Carcinoma cell line)
Lane 6 : Skeletal Muscle (Human) Tissue Lysate - adult normal tissue
Lane 7 : Heart (Human) Tissue Lysate - adult normal tissue

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (Alexa Fluor® 790) (ab175783) at 1/10000 dilution

Predicted band size: 43 kDa
Observed band size: 42 kDa
why is the actual band size different from the predicted?

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 5% Milk before being incubated with ab14128 overnight at 4°C. Antibody binding was detected using ab175783 at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.



Immunocytochemistry/ Immunofluorescence - Anti-Actin antibody [C4] (ab14128)

ab14128 staining Actin in HeLa cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab14128 at 5µg/ml and ab6046 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an Goat anti-Mouse Alexa Fluor 488 secondary (ab150117) at 2 µg/ml (shown in green) and Goat anti-Rabbit Alexa Fluor 594 secondary (ab150084) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.
Negative controls: 1– Rabbit primary and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.

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