abcam

Product datasheet

Anti-beta Actin antibody [mAbcam 8226] - Loading Control ab8226

*** * 136 Abreviews 2899 References 7 Images

Overview

Product name Anti-beta Actin antibody [mAbcam 8226] - Loading Control

Description Mouse monoclonal [mAbcam 8226] to beta Actin - Loading Control

Host species Mouse

Specificity Does not cross-react with adult cardiac, smooth, or skeletal muscle actin. The immunogen used

for this product shares 77% homology with Gamma actin/actin cytoplasmic 2. Cross-reactivity with

this protein has not been confirmed experimentally.

Tested applications Suitable for: ICC/IF, IHC-P, WB

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Sheep, Rabbit, Horse, Chicken, Guinea pig, Cow, Dog, Pig, Monkey,

Zebrafish, African green monkey, Chinese hamster, Armenian hamster

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as ab13772)

Positive control WB, ICC/IF: HeLa, Jurkat, A431, HEK293, NIH 3T3, PC12 cells. IHC-P: Human colon (FFPE),

Rat colon (FFPE) tissue. Hela cells

General notes Western blot protocol advice:

We recommend blocking with 2-5% BSA as we have found that use of 5% milk significantly reduces the band intensity for beta actin. Please see the comparison data in the images section. If milk block is required, we recommend using <u>ab8224</u> mouse monoclonal [mAbcam 8224] to beta actin. Contact our Scientific Support team for more information or advice.

This antibody clone [mAbcam 8226] is manufactured by Abcam.

If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact <u>orders@abcam.com</u> or you can find further information <u>here</u>.

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

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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 or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine

Purity Protein G purified

ClonalityMonoclonalClone numbermAbcam 8226MyelomaSp2/0-Ag14

lsotype lgG1 **Light chain type** kappa

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab8226 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	★★★★ ★ (17)	Use a concentration of 5 µg/ml.
IHC-P	★★★★ (1)	Use a concentration of 0.1 - 0.5 μ g/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	*** (100)	Use a concentration of 1 µg/ml. Predicted molecular weight: 42 kDa.Can be blocked with Human beta Actin peptide (ab13772). We recommend blocking using 2-5% BSA as we have found that use of 5% milk significantly reduces the band intensity for beta actin. Please refer to the images section for the blocking comparison data.

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 ACTB are a cause of dystonia juvenile-onset (DYTJ) [MIM:607371]. DYTJ is a form of

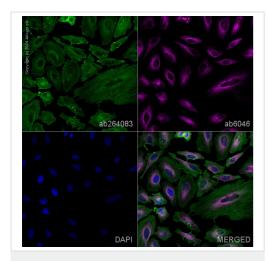
dystonia with juvenile onset. Dystonia is defined by the presence of sustained involuntary muscle contraction, often leading to abnormal postures. DYTJ patients manifest progressive, generalized,

 $\ dop a\hbox{-unresponsive dystonia, developmental malformations and sensory hearing loss.$

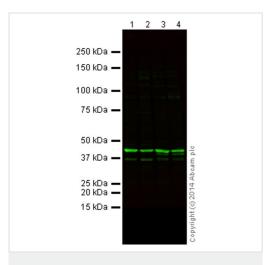
Sequence similaritiesBelongs to the actin family.

Post-translational ISGylated.

Images



Immunocytochemistry/ Immunofluorescence - Antibeta Actin antibody [mAbcam 8226] - Loading Control (ab8226)



Western blot - Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226)

This data was developed using the same antibody clone in a different buffer formulation containing only PBS (ab264083). ab264083 staining beta Actin in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab264083 at 5µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

All lanes : Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226) at 1/1000 dilution

Lane 1: A431 (Human epidermoid carcinoma cell line) Whole Cell Lysate

Lane 2: HEK293 (Human embryonic kidney cell line) Whole Cell

Lane 3: NIH 3T3 (Mouse embryo fibroblast cell line) Whole Cell Lysate

Lane 4 : PC12 (Rat adrenal gland pheochromocytoma cell line)
Whole Cell Lysate

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: 42 kDa **Observed band size:** 42 kDa

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 ab8226 overnight at 4°C. Antibody binding was detected using **Goat Anti-Mouse IgG H&L (Alexa Fluor® 790) (ab175783)** at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.

Secondary only

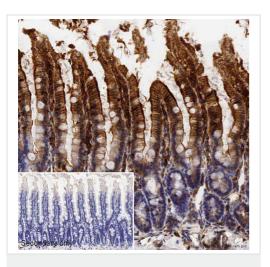
equipment 2-244 5 Automost

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226)

IHC image of ab8226 staining beta Actin in human colon formalin fixed paraffin embedded tissue sections*, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab8226, 0.1µg/ml working concentration, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset). For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta Actin antibody
[mAbcam 8226] - Loading Control (ab8226)

IHC image of ab8226 staining beta Actin in rat colon formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab8226, 0.5µg/ml working concentration, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset). For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226)

Lane 1: Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226) at 1/1000 dilution

Lane 2: Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226) at 1/10000 dilution

Lanes 3-4: Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226) at 1/500 dilution

Lanes 1-2: HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate

Lane 3: HEK293 (Human epithelial cell line from embryonic kidney) cell lysate

Lane 4: NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Rabbit Anti-Mouse IgG H&L (HRP) (ab6728) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 42 kDa

Exposure time: 10 seconds

All lanes: Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226) at 1 µg/ml

Lane 1: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 3: A431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 4: HEK293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 5: HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

240 kDa -165 kDa -125 kDa 93 kDa -57 kDa -42 kDa -31 kDa -Copyright (c) 2011 Abcam 24 kDa -18 kDa -15 kDa -8 kDa

Western blot - Anti-beta Actin antibody [mAbcam

8226] - Loading Control (ab8226)

Secondary

All lanes: HRP-conjugated goat anti-mouse IgG at 1/5000 dilution

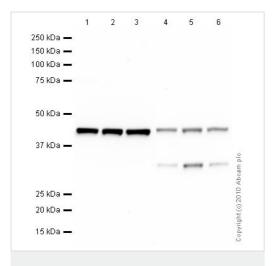
Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 10 seconds

Western blot image using the Optiblot Reducing Electrophoresis Kit - 10 x 10 cm (4-20%) with the Prism Ultra Protein Ladder (**ab116028**) 5μ I used. We recommend using our ECL substrate kit (**ab65623**).



Western blot - Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226)

Lanes 1-3: Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226) at 1 µg/ml (5% BSA BLOCK)

Lanes 4-6: Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226) at 1 µg/ml (5% MILK BLOCK)

Lanes 1 & 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lanes 2 & 5: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lanes 3 & 6 : NIH/3T3 (Mouse embryo fibroblast cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

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

Exposure time: 8 minutes

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