


Product datasheet

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

★★★★★ 130 Abreviews 875 References 9 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, ICC, Flow Cyt, IHC-FrFI, IHC-P, IHC-Fr, IP, WB
Species reactivity	Reacts with: Mouse, Rat, Rabbit, Horse, Chicken, Cow, Dog, Human, Pig, Zebrafish, African green monkey, Chinese hamster, Armenian hamster Predicted to work with: Sheep, Guinea pig 
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 1 - 100 of Human beta Actin. Read Abcam's proprietary immunogen policy (Peptide available as ab13772 .)
Positive control	WB, ICC/IF, Flow Cyt: HeLa, Jurkat, A431, HEK293, NIH 3T3, PC12 cells. IHC-P: Human colon (FFPE), Rat colon (FFPE).
General notes	<u>Western blot protocol advice:</u> For best results in Western blot, we recommend using 2-5% BSA for blocking not milk since we have found that using 5% milk significantly reduces the band intensity for beta actin. Please see the comparison data in the images section. If milk block is needed, we recommend using ab8224 mouse monoclonal [mAbcam 8224] to beta actin. Our Technical team (technical@abcam.com) will be happy to provide further information and 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 orders@abcam.com or you can find further information here . Abcam recommended secondaries - Goat Anti-Mouse HRP (ab205719) and Goat Anti-Mouse Alexa Fluor® 488 (ab150113). See other anti-mouse secondary antibodies that can be used with this antibody.

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	IgG fraction
Clonality	Monoclonal
Clone number	mAbcam 8226
Myeloma	Sp2/0-Ag14
Isotype	IgG1
Light chain type	kappa

Applications

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	★★★★☆	Use a concentration of 5 µg/ml.
ICC	★★★★★	Use at an assay dependent concentration.
Flow Cyt	★★★★★	Use at an assay dependent concentration. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IHC-FrFI	★★★★☆	Use at an assay dependent concentration.
IHC-P	★★★★☆	Use a concentration of 0.1 - 0.5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IHC-Fr	★★★★★	1/500.
IP	★★★★★	1/100.
WB	★★★★★	1/500 - 1/10000. 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, dopa-unresponsive dystonia, developmental malformations and sensory hearing loss.

Sequence similarities

Belongs to the actin family.

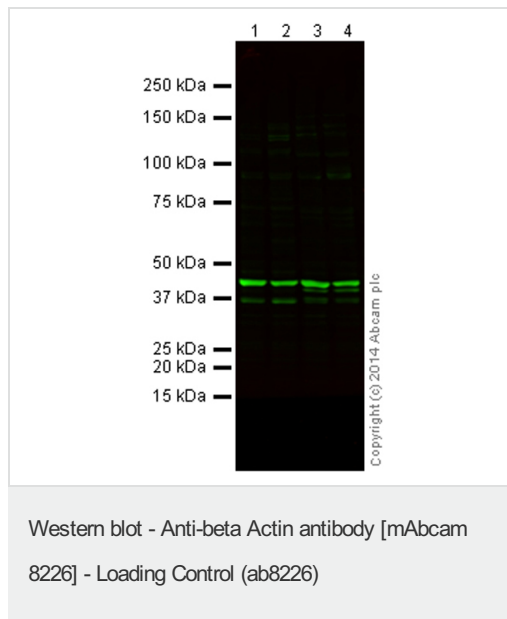
Post-translational modifications

ISGylated.

Cellular localization

Cytoplasm > cytoskeleton. Localized in cytoplasmic mRNP granules containing untranslated mRNAs.

Images



All lanes : Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226) 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 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

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

Lysates/proteins at 20 µg per lane.

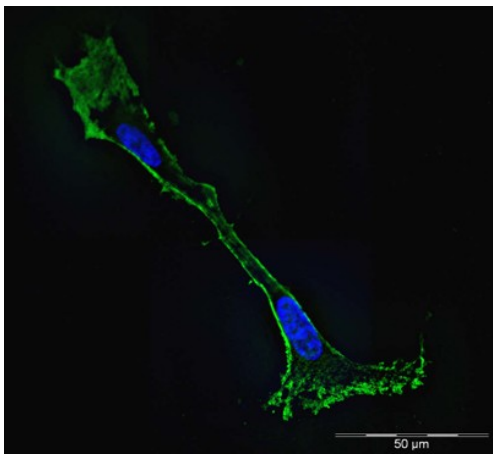
Secondary

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.

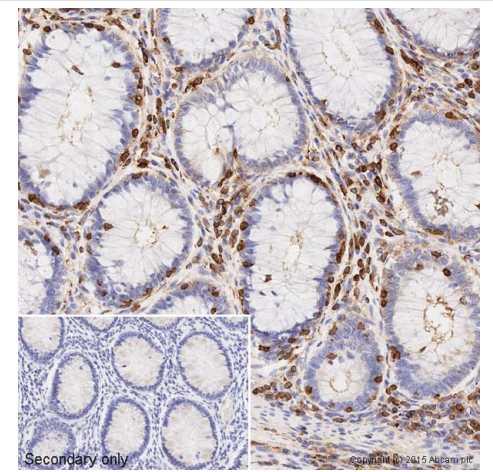


Immunocytochemistry/ Immunofluorescence - Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226)

Immunofluorescence using ab8226 at 5μg/ml incubated for 1 hour on Rat Colon Cancer cells.

Cells were fixed with ice-cold methanol for 5 mins, then for all following steps, permeabilised in TBS-T for 30 mins, blocked with 5% BSA for 30 mins and then washed in TBS-T. Secondary antibody was Alexa Fluor 488 goat anti-mouse IgG at 1/1000 incubated for 1 hour. Cells were counterstained with DAPI. Image at 400X magnification. All incubations were at room temperature.

The beta actin fibres can be seen arrayed around the edge of the cells.

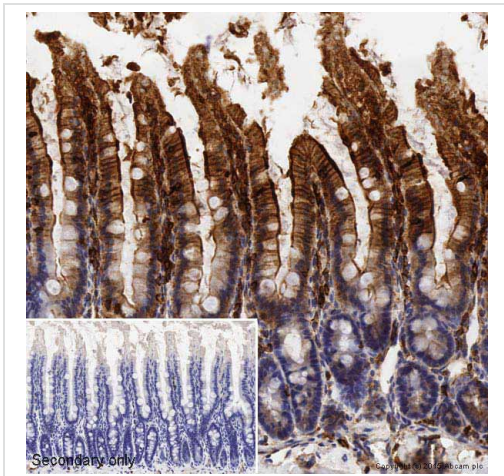


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226)

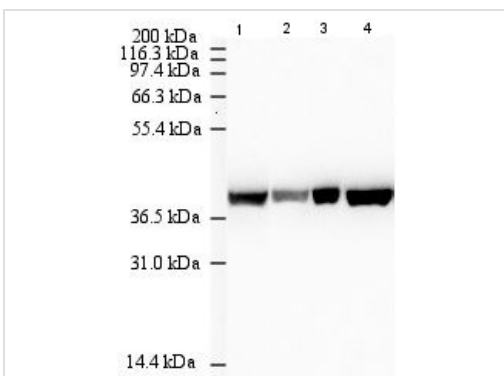
IHC image of ab8226 staining beta Actin in human colon formalin fixed paraffin embedded tissue sections*, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab8226, 0.1 μg/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.

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



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



Western blot - 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.

- 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 cell lysate
- Lane 3 :** HEK293 cell lysate
- Lane 4 :** NIH 3T3 mouse cell lysate

Lysates/proteins at 20 µg per lane.

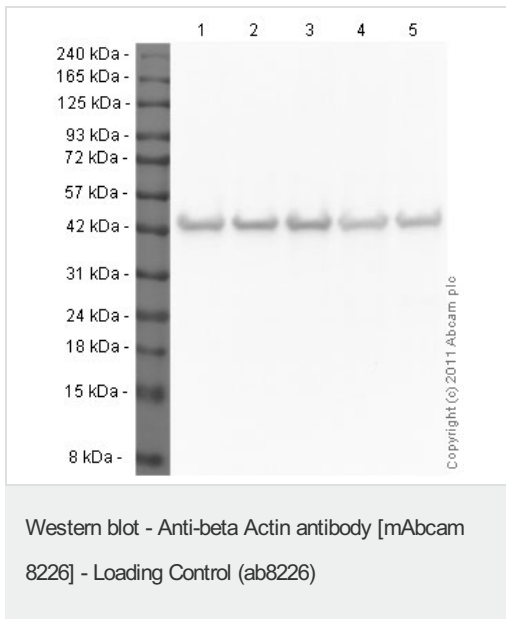
Secondary

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 carcinoma cell line) Whole Cell Lysate

Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

Lane 3 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 4 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lane 5 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/10000 dilution

Developed using the ECL technique.

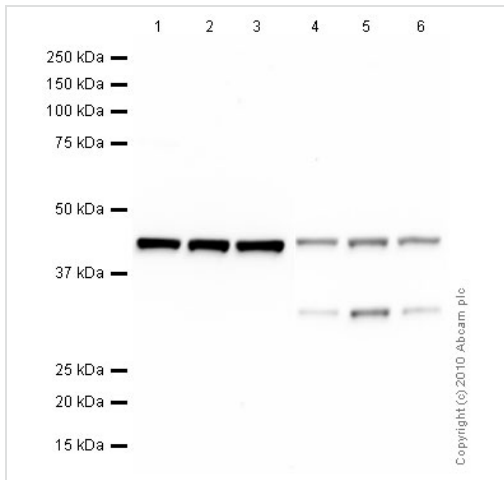
Performed under reducing conditions.

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%) ([ab119220](#)) with the Prism Ultra Protein Ladder ([ab116028](#)) 5µl 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 carcinoma cell line) Whole Cell Lysate

Lanes 2 & 5 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

Lanes 3 & 6 : NIH 3T3 (Mouse embryonic 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

Anti-beta Actin antibody [mAbcam 8226] - Loading Control (ab8226) at 0.5 µg/ml + HeLa cell lysate

Secondary

Goat polyclonal to mouse IgG H&L (HRP) at 1/5000 dilution

Performed under non-reducing conditions.

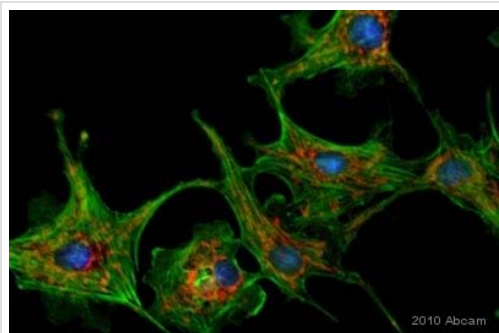
Predicted band size: 42 kDa

Observed band size: 42 kDa

Exposure time: 30 seconds



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



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

This image is a courtesy of Anonymous Abreview

ab8226 staining beta Actin in human gastric epithelial AGS cells by Immunocytochemistry/ Immunofluorescence. Cells were fixed in formaldehyde. Permeabilization and blocking was carried out using 5% BSA containing 0.025% Triton X in TBS for 1 hour at 23°C. Primary antibody was used at 5µg/ml for 1 hour at 23°C. An Alexa Fluor 488® conjugated goat polyclonal to mouse IgG was used as the secondary antibody at a 1/1000 dilution.

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