

## Product datasheet

# Anti-beta Actin antibody [mAbcam 8224] - Loading Control ab8224

★★★★★ 28 Abreviews 226 References 11 Images

### Overview

<b>Product name</b>	Anti-beta Actin antibody [mAbcam 8224] - Loading Control
<b>Description</b>	Mouse monoclonal [mAbcam 8224] to beta Actin - Loading Control
<b>Host species</b>	Mouse
<b>Specificity</b>	Recognises a single band at 42kD representing beta 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.
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt, WB, IHC-P, ICC/IF
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Rabbit, Chicken, Cow, Cat, Dog, Human, Pig, Xenopus laevis, Drosophila melanogaster, Schizosaccharomyces pombe, Chinese hamster, Other species
<b>Immunogen</b>	Synthetic peptide conjugated to KLH derived from within residues 1 - 100 of Human beta Actin. Read Abcam's proprietary immunogen policy (Peptide available as <a href="#">ab13772</a> .)
<b>Positive control</b>	WB: A431; HEK293; NIH3T3; PC12 whole cell lysates; Xenopus embryo lysate; Drosophila lysate; S. pombe lysate. Flow Cyt: HeLa cells. ICC/IF: Panc-1 cells; Human fibroblasts. IHC/P: Human colon (FFPE)
<b>General notes</b>	<p>This monoclonal antibody to beta actin works well as a protein loading control in Western blot for a broad range of species including Xenopus, Drosophila and S. pombe.</p> <p>This antibody clone [mAbcam 8224] is manufactured by Abcam.</p> <p>If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact <a href="mailto:orders@abcam.com">orders@abcam.com</a> or you can find further information <a href="#">here</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C or -80°C. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
<b>Purity</b>	IgG fraction

<b>Primary antibody notes</b>	This clone works well as a loading control for <i>Xenopus</i> , <i>Drosophila</i> , <i>S. cerevisiae</i> and <i>S.pombe</i> . We recommend using ab8224 instead of <a href="#">ab8226</a> for these species.
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	mAbcam 8224
<b>Myeloma</b>	Sp2/0-Ag14
<b>Isotype</b>	IgG1
<b>Light chain type</b>	kappa

## Applications

Our [Abpromise guarantee](#) covers the use of **ab8224** in the following tested applications.

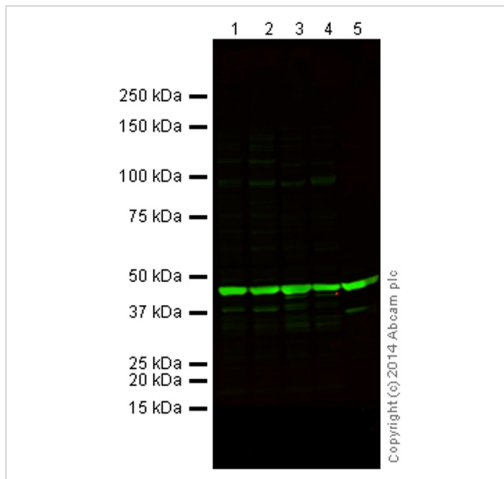
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		Use 1µg for 10 <sup>6</sup> cells. <a href="#">ab18392</a> - Mouse monoclonal IgG3, is suitable for use as an isotype control with this antibody.
WB	★★★★★	Use a concentration of 1 µg/ml. Detects a band of approximately 42 kDa (predicted molecular weight: 42 kDa). Can be blocked with <a href="#">Human beta Actin peptide (ab13772)</a> . This antibody has been designed for use as a loading control and is ideal for this purpose. Block membrane for 1 hr in 5%BSA. Incubate antibody in TBST for one hour or more.
IHC-P	★★★★★	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★★	Use a concentration of 1 µg/ml.

## Target

<b>Function</b>	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.
<b>Involvement in disease</b>	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.
<b>Sequence similarities</b>	Belongs to the actin family.
<b>Post-translational modifications</b>	ISGylated.
<b>Cellular localization</b>	Cytoplasm > cytoskeleton. Localized in cytoplasmic mRNP granules containing untranslated mRNAs.

## Images



Western blot - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

**All lanes** : Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224) at 1 µg/ml

**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

**Lane 5** : Skeletal Muscle (Human) Tissue Lysate - adult normal tissue

Lysates/proteins at 20 µg per lane.

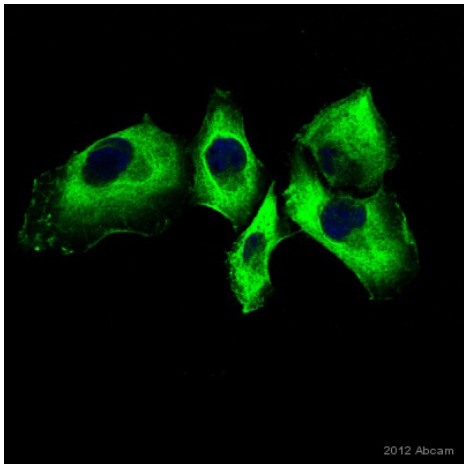
### Secondary

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

**Predicted band size:** 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 ab8224 overnight at 4°C. Antibody binding was detected using a goat anti-mouse Alexa Fluor 790 (ab175783) at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.

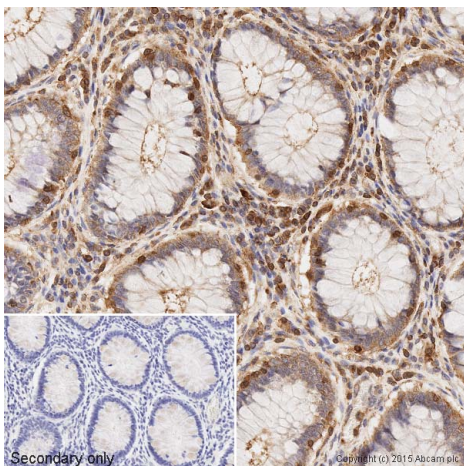


Immunocytochemistry/ Immunofluorescence - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

This image is courtesy of an Abreview submitted by Armen Petrosyan

Immunofluorescence analysis of Human Panc-1 cells, staining beta Actin with ab8224.

Cells were fixed with formaldehyde and blocked with 1% donkey serum in PBST for 1 hour at 22°C. Samples were incubated with primary antibody (1/75 in 1% donkey serum in PBST) for 1 hour at 22°C. A DyLight®488-conjugated donkey anti-mouse polyclonal IgG (1/200) was used as the secondary antibody.

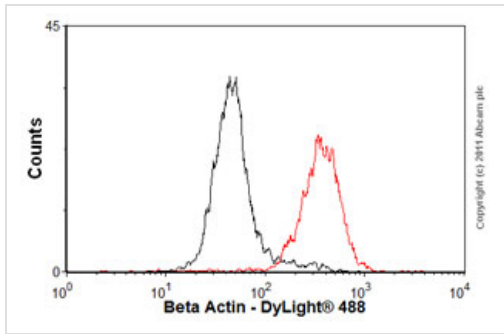


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

IHC image of ab8224 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 ab8224, 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).

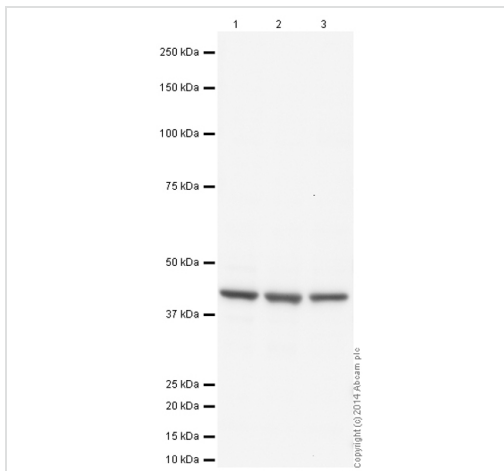
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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



Flow Cytometry - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

Overlay histogram showing HeLa cells stained with ab8224 (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 (ab8224, 1µg/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was a goat anti-mouse DyLight® 488 (IgG; H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was Mouse IgG3 [MG3-35] (ab18394, 1µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed.



Western blot - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

**All lanes :** Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224) at 1 µg/ml

**Lane 1 :** HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

**Lane 2 :** NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

**Lane 3 :** PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes :** Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/50000 dilution

Performed under reducing conditions.

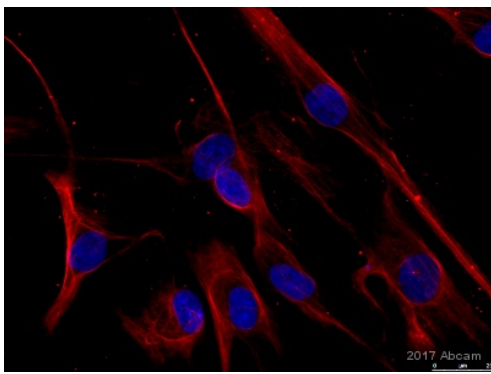
**Predicted band size:** 42 kDa

**Observed band size:** 42 kDa

**Exposure time:** 3 minutes

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 2% Bovine Serum Albumin before being incubated with ab8224 overnight at

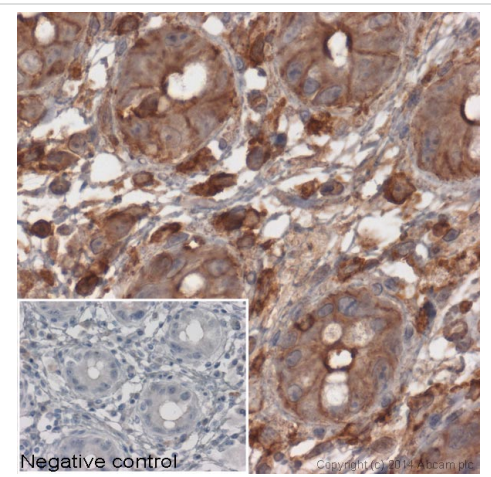
4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution [ab133406](#)



Immunocytochemistry/ Immunofluorescence - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

This image is courtesy of an anonymous abreview.

Paraformaldehyde-fixed human fibroblasts cells stained for beta Actin (red) using ab8224 at 1/200 dilution in ICC/IF.

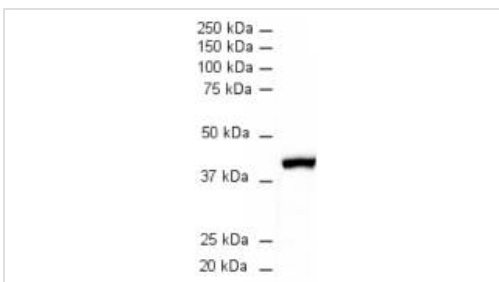


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

IHC image of beta actin staining in human colon formalin fixed paraffin embedded tissue section\*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins. The section was incubated with ab8224, 3µg/ml overnight at +4°C. A goat [anti-mouse HRP-conjugated secondary antibody \(ab6789, 1/2000 dilution\)](#) was used for 1hr at room temperature. The section was counterstained with haematoxylin and mounted with DPX.

The inset negative control image is secondary-only at 1/500 dilution.

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



Western blot - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224) + Xenopus embryo lysate at 20 µg

### Secondary

Rabbit Anti-Mouse IgG H&L (HRP) ([ab6728](#))

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 42 kDa

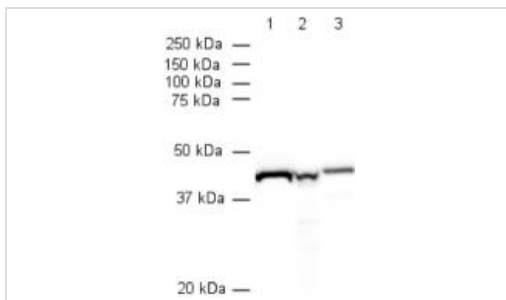
ab8224 used on *Xenopus* embryo lysate (20 µg of lysate/lane).

### Secondary

Rabbit polyclonal [anti-mouse HRP](#) was used as the secondary antibody ([ab6728](#)) and developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size :** 42kD



Western blot - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

**All lanes :** Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224) at 1 µg/ml

**Lane 1 :** *Drosophila* lysate

**Lane 2 :** *S. pombe* lysate

**Lane 3 :** *S. cerevisiae* lysate (Actin 1 - please see note)

Lysates/proteins at 20 µg per lane.

### Secondary

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

Developed using the ECL technique.

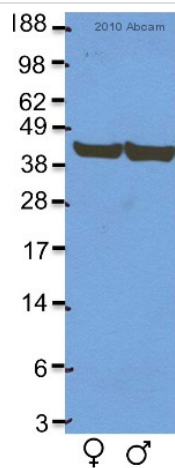
Performed under reducing conditions.

**Predicted band size:** 42 kDa

**Observed band size:** 42 kDa

Note: although *S. cerevisiae* is not known to express beta Actin, Abcam believes that the band on lane 3 corresponds to Actin 1 (Swissprot ID: P60010, based on sequence similarity).

Secondary antibody - rabbit [anti-mouse HRP](#) ([ab6728](#))



Western blot - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

This image is courtesy of an anonymous Abreview

**All lanes :** Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224) at 1/1000 dilution

**Lane 1 :** Fruit fly (*Drosophila melanogaster*) whole cell lysate - Female

**Lane 2 :** Fruit fly (*Drosophila melanogaster*) whole cell lysate - Male

Lysates/proteins at 100 µg per lane.

**Secondary**

**All lanes :** An HRP-conjugated Sheep polyclonal to mouse IgG at 1/10000 dilution

Developed using the ECL technique.

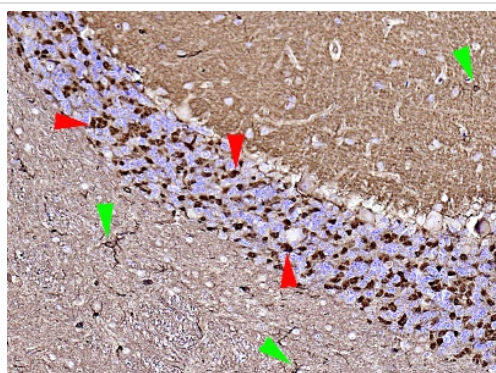
Performed under reducing conditions.

**Predicted band size:** 42 kDa

**Exposure time:** 2 minutes

**Blocking step:** 5% Milk for 1 hour at 20°C.

Immunohistochemical detection of beta Actin using antibody [mAbcam 8224] - Loading Control on formaldehyde-fixed paraffin-embedded rat cerebellum sections. Antigen retrieval step: heat mediated Citric acid pH6 buffer. Permeabilization: No. Blocking step: 1% BSA for 10 mins @ rt°C. Primary antibody dilution 1/1000 for 2 hours in TBS/BSA/azide. Secondary Antibody: anti Mouse Igs conjugated to biotin (1/200). beta Actin appears to be particularly enriched not only in the glomeruli of the Granule cell layer (indicated by red arrowheads ) but also in Microglia (indicated by green arrowheads); All positive microglia appear to be ramified thus not presumed to be activated.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Actin antibody [mAbcam 8224] - Loading Control (ab8224)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom



## **Our Abpromise to you: Quality guaranteed and expert technical support**

---

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
  
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

## **Terms and conditions**

---

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors