Product name: Anti-beta Actin antibody [mAbcam 8224] - Loading Control ab8224

Description: Mouse monoclonal [mAbcam 8224] to beta Actin - Loading Control

Host species: Mouse

Specificity: 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.

Tested applications: Suitable for: WB, IHC-P, Flow Cyt (Intra)

Species reactivity: Reacts with: Mouse, Rat, Human, Xenopus laevis, Drosophila melanogaster, Schizosaccharomyces pombe

Predicted to work with: Rabbit, Chicken, Cow, Cat, Dog, Pig, Chinese hamster, Other species

Immunogen: Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as ab13772)

Positive control: WB: A431; HEK293; NIH3T3; PC12 whole cell lysates; Xenopus embryo lysate; Drosophila lysate; S. pombe lysate. Flow Cyt (Intra): HeLa cells. ICC/IF: Panc-1 cells; Human fibroblasts. IHC/P: Human colon (FFPE)

General notes: 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.

This antibody clone [mAbcam 8224] 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.

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.
Form: Liquid

Storage instructions: Shipped at 4°C. Store at +4°C short term (1-2 weeks). 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

Primary antibody notes: This clone works well as a loading control for Xenopus, Drosophila, S. cerevisiae and S.pombe. We recommend using ab8224 instead of ab8226 for these species.

Clonality: Monoclonal

Clone number: mAbcam 8224

Myeloma: Sp2/0-Ag14

Isotype: IgG1

Light chain type: kappa

Applications

The Abpromise guarantee: 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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★★★★ (22)</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 42 kDa (predicted molecular weight: 42 kDa). Can be blocked with Human beta Actin peptide (ab13772). 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.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★★★ (7)</td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>Flow Cyt (Intra)</td>
<td></td>
<td>Use 1µg for 10^6 cells. ab18392 - Mouse monoclonal IgG3, is suitable for use as an isotype control with this antibody.</td>
</tr>
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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**

**Western blot - 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.
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

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/1x10⁶ 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/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.
**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.
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) + Xenopus embryo lysate at 20 µg

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

**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 melanogastor) whole cell lysate - Female

**Lane 2**: Fruit fly (Drosophila melanogastor) 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 IgG 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.

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