## Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-beta Actin antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit polyclonal to beta Actin</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Specificity</td>
<td>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.</td>
</tr>
<tr>
<td>Tested applications</td>
<td>Suitable for: IHC-Fr, IP, WB, ICC, Flow Cyt, IHC-FrFl, IHC-P, ICC/IF, ELISA</td>
</tr>
<tr>
<td>Species reactivity</td>
<td>Reacts with: Mouse, Rat, Sheep, Rabbit, Chicken, Guinea pig, Cow, Dog, Human, Pig, Xenopus laevis, Drosophila melanogaster, Fish, Monkey, Zebrafish, Rhesus monkey, Chinese hamster</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human beta Actin aa 1-100. The exact sequence is proprietary. (Peptide available as ab28691, ab13772)</td>
</tr>
<tr>
<td>Positive control</td>
<td>WB: Mouse brain tissue lysate and HeLa, A431, HEK293, NIH3T3 and PC12 whole cell lysates. IHC-P: Normal human colon and normal human liver tissues. ICC/IF: NIH3T3 and SV40LT-SMC cells.</td>
</tr>
<tr>
<td>General notes</td>
<td>For Western blotting, do not use milk for blocking. Our labs have extensively tested the blocking conditions for this antibody and recommend using 2 % BSA for 1 hour. Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077). See other anti-rabbit secondary antibodies that can be used with this antibody.</td>
</tr>
</tbody>
</table>

## Properties

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage instructions</td>
<td>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.</td>
</tr>
<tr>
<td>Storage buffer</td>
<td>Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
</tbody>
</table>
Isotype

IgG

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.

Applications

Our Abpromise guarantee covers the use of ab8227 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>
</tr>
</thead>
<tbody>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000 - 1/5000. Recommend Goat Anti-Rabbit IgG H&amp;L (HRP) (ab6721) secondary antibody. A stronger signal is observed in western blot when using 2% BSA as the blocking agent.</td>
</tr>
<tr>
<td>ICC</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IHC-FrFl</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 0.2 µg/ml. We recommend Goat Anti-Rabbit IgG H&amp;L (Biotin) (ab6720) secondary antibody or Goat Anti-Rabbit IgG Fc (HRP) (ab97200) secondary antibody.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>ELISA</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000.</td>
</tr>
</tbody>
</table>

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.
Immunocytochemistry/ Immunofluorescence - Anti-beta Actin antibody (ab8227)

ab8227 staining beta Actin in SV40LT-SMC cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% 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 ab8227 at 1μg/ml (detected with ab150081, Alexa Fluor® 488 Goat anti-Rabbit, 1μg/ml, shown in green); and ab195889, Mouse monoclonal [DM1A] to alpha Tubulin - Microtubule Marker (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Actin antibody (ab8227)

IHC image of ab8227 staining beta Actin in rat small intestine formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with EDTA (epitope retrieval solution 2) for 20 mins. The section was then incubated with ab8227, 0.2μg/ml, 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 (ab8227)**

**All lanes**: Anti-beta Actin antibody (ab8227) 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-Rabbit IgG H&L (Alexa Fluor® 790) (ab175781) at 1/10000 dilution

**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 ab8227 overnight at 4°C. Antibody binding was detected using Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (ab175781) secondary antibody at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.
ab8227 staining beta Actin in NIH3T3 cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% 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 ab8227 at 1μg/ml (shown in green) and ab195889, Mouse monoclonal [DM1A] to alpha Tubulin - Microtubule Marker (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

IHC image of beta actin staining in a section of formalin-fixed paraffin-embedded normal human colon*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins. The section was then incubated with ab8227, 1/1000 dilution, for 15 mins at room temperature. A goat anti-rabbit biotinylated secondary antibody (ab6720, 1/1000 dilution) was used to detect the primary, and visualized using an HRP conjugated ABC system. Streptavidin HRP was used, ab7403 at a 1/10000 dilution. DAB was used as the chromogen (ab103723), diluted 1/100 and incubated for 10min at room temperature. The section was then counterstained with haematoxylin and mounted with DPX.

The inset negative control image is taken from an identical assay without primary antibody.

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
All lanes: Anti-beta Actin antibody (ab8227) at 1 µg/ml

Lane 1: HeLa whole cell lysate (blocked with 2% BSA)
Lane 2: NIH3T3 whole cell lysate (blocked with 2% BSA)
Lane 3: Rat Liver tissue lysate (blocked with 2% BSA)
Lane 4: HeLa whole cell lysate (blocked with 3% Milk)
Lane 5: NIH3T3 whole cell lysate (blocked with 3% Milk)
Lane 6: Rat Liver tissue lysate (blocked with 3% Milk)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 45 kDa why is the actual band size different from the predicted?

Exposure time: 10 seconds

Lanes 1-3: Blocked with 2% BSA
Lanes 4-6: Blocked with 3% Milk

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 or 3% Milk before being incubated with ab8227 overnight at 4°C. Antibody binding was detected using an anti rabbit HRP secondary antibody, and visualised using ECL development solution ab133406
**All lanes**: Anti-beta Actin antibody (ab8227) at 0.1 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**: Liver (Rat) Tissue Lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Performed under reducing conditions.

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

**Exposure time**: 1 minute

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 ab8227 overnight at 4°C. Antibody binding was detected using an anti-rabbit HRP secondary antibody, and visualised using ECL development solution ab133406
All lanes: Anti-beta Actin antibody (ab8227) at 1/1000 dilution

Lane 1: HeLa nuclear lysate
Lane 2: HeLa whole cell lysate
Lane 3: A431 cell lysate
Lane 4: Jurkat cell lysate
Lane 5: HEK 293 cell lysate
Lane 6: HeLa nuclear lysate with Human beta Actin peptide (ab13772) at 1 µg/ml
Lane 7: HeLa whole cell lysate with Human beta Actin peptide (ab13772) at 1 µg/ml
Lane 8: A431 cell lysate with Human beta Actin peptide (ab13772) at 1 µg/ml
Lane 9: Jurkat cell lysate with Human beta Actin peptide (ab13772) at 1 µg/ml
Lane 10: HEK 293 cell lysate with Human beta Actin peptide (ab13772) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab6721) at 1/5000 dilution

Observed band size: 41.7 kDa why is the actual band size different from the predicted?

Exposure time: 5 seconds
Western blot - Anti-beta Actin antibody (ab8227)

All lanes: Anti-beta Actin antibody (ab8227) at 1/1000 dilution

Lane 1: HeLa cells (Human)
Lane 2: 3T3 cells (Mouse)
Lane 3: Fish Liver
Lane 4: Rabbit Liver
Lane 5: MDCK cells (Dog)
Lane 6: EBTr cells (Cow)
Lane 7: SL-29 cells (Chicken)
Lane 8: CHO cells (Chinese Hamster)
Lane 9: Xenopus embryo

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab6721) at 1/5000 dilution

Observed band size: 41.7 kDa. Why is the actual band size different from the predicted?

Exposure time: 30 seconds
IHC image of beta Actin staining in normal human colon, formalin-fixed and paraffin-embedded tissue*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins. The section was incubated with ab8227, 3µg/ml overnight at +4°C. A anti-rabbit HRP secondary antibody (Ab97200, 1/200 dilution) was used for 1hr at room temperature. The section was counterstained with haematoxylin and mounted with DPX.

The inset negative control image is taken from an identical assay without primary antibody.

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

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