Product name: Anti-beta Actin antibody [AC-15] ab6276

Description: Mouse monoclonal [AC-15] to beta Actin

Host species: Mouse

Tested applications: Suitable for: ICC/IF, IHC-FoFr, ICC, IHC-P, IHC-Fr, Indirect ELISA, WB, ELISA

Species reactivity: Reacts with: Mouse, Rat, Sheep, Rabbit, Chicken, Guinea pig, Hamster, Cow, Cat, Dog, Human, Carp, African green monkey, Opossum, Chinese hamster, Common marmoset, Meriones unguiculatus

Does not react with: Drosophila melanogaster, Dictyostelium discoideum

Immunogen: Synthetic peptide corresponding to beta Actin aa 1-14 (N terminal) conjugated to Keyhole Limpet Haemocyanin (KLH). Slightly modified β-cytoplasmic actin N-terminal peptide, Ac-Asp-Asp-Ile-Ala-Ala-Leu-Val-Ile-Asp-Asn-Gly-Ser-Gly-Lys, conjugated to KLH.

Sequence: DDDIAALVIDNGSGK

Epitope: N-terminal of the beta isoform of actin.

Positive control: ICC/IF: SV40LT-SMC cells WB: HAP1, HeLa, Jurkat, A431, HEK-293, COS-7, NIH/3T3, PC-12, Rat2, CHO, MDBK and MDCK cell lysates. IHC-Fr: Human stomach tissue. ICC/IF: SV40LT-SMC cells.


Properties:

Form: Liquid


Storage buffer: pH: 7.4
Preservative: 0.097% Sodium azide
Constituent: PBS
Purity: Affinity purified

Purification notes: Purified from hybridoma cell culture.

Clonality: Monoclonal

Clone number: AC-15

Isotype: IgG1

Applications

Our Abpromise guarantee covers the use of **ab6276** in the following tested applications.

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

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 5 µg/ml.</td>
</tr>
<tr>
<td>IHC-FoFr</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>IHC-Fr</td>
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<td>Use at an assay dependent concentration.</td>
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<tr>
<td>Indirect ELISA</td>
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<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td>1/5000 - 1/16000. Predicted molecular weight: 42 kDa.</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</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

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**Lane 1**: Wild-type HAP1 cell lysate (20 µg)

**Lane 2**: Beta actin knockout HAP1 cell lysate (20 µg)

**Lanes 1 and 2**: Merged signal (red and green). Green - beta actin, ab6276 observed at 42 kDa. Red - loading control, ab181602 observed at 37 kDa.

Ab6276 was shown to specifically react with beta actin in wild-type HAP1 cells. No band was observed when beta actin knockout samples were used. Wild-type and beta actin knockout samples were subjected to SDS-PAGE. ab6276 (beta actin) and ab181602 (loading control to GAPDH) were diluted 1/5000 and 1/10 000 and incubated overnight at 4°C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.
ab6276 staining beta Actin in SV40LT-SMC cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab6276 at a working concentration of 5μg/ml and ab190573, Rabbit monoclonal [EP1332Y] to alpha Tubulin (Alexa Fluor® 647, shown in red) at 1/250 overnight at +4°C, followed by a further incubation at room temperature for 1h with an anti-mouse AlexaFluor® 488 (ab150117) at 2 μg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

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

Immunohistochemical frozen analysis of acetone-fixed human stomach tissue labeling beta Actin with ab6276 at 1/5000 dilution, followed by secondary antibody.
Western Blot of ab6276 (used as loading control) with whole tissue lysate of grey matter from BA20 (temporal cortex). Ab6276 was diluted 1/50000 and incubated with the sample for 16 hours at 4°C. 5 µg of lysate was loaded onto the gel, which was blocked with 5% milk for 1 hour at 20°C. An Alexa Fluor® 680 conjugated goat anti-mouse antibody, diluted 1/5000, was used as the secondary.

Bands below actin in image are synaptophysin (SYN).
Western blot - Anti-beta Actin antibody [AC-15] (ab6276)

All lanes: Anti-beta Actin antibody [AC-15] (ab6276) at 1 µg/ml

Lane 1: HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2: Jurkat (human T cell leukemia cell line from peripheral blood) cell lysate
Lane 3: COS-7 (african green monkey kidney fibroblast-like cell line) cell lysate
Lane 4: NIH/3T3 (mouse embryonic fibroblast cell line) cell lysate
Lane 5: PC-12 (rat adrenal gland pheochromocytoma cell line) cell lysate
Lane 6: Rat2 (rat fibroblast cell line) cell lysate
Lane 7: CHO (chinese hamster ovary cell line) cell lysate
Lane 8: MDBK (bovine kidney cell line) cell lysate
Lane 9: MDCK (canine kidney cell line) cell lysate

Secondary
All lanes: Goat Anti-Mouse IgG-Peroxidase

Developed using the ECL technique.

Predicted band size: 42 kDa
**All lanes**: Anti-beta Actin antibody [AC-15] (ab6276) at 1/5000 dilution

**Lane 1**: HeLa nuclear  
**Lane 2**: HeLa whole cell lysate  
**Lane 3**: A431 cell lysate  
**Lane 4**: Jurkat cell lysate  
**Lane 5**: HEK293 cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: Alexa Fluor anti mouse at 1/5000 dilution

Performed under reducing conditions.

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

MDCK cells induced with increasing amounts of doxycycline to control expression of the gene of interest. All cells were normalized for loading with an albumin protein standard assay. Anti-beta actin (ab6276) was used at a concentration of 1:5000 in a milk blocking solution. B-actin blotting confirms the albumin assay in showing that an equal amount of lysate was loaded in each lane.

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