Product datasheet

Anti-beta Actin antibody [AC-15] ab6276

Overview

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

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

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.

General notes

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
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>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<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>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>Indirect</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ELISA</td>
<td></td>
<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>
</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.
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).

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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