Product datasheet

Anti-Actin antibody - Loading Control ab1801

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Overview

Product name Anti-Actin antibody - Loading Control
Description Rabbit polyclonal to Actin - Loading Control
Host species Rabbit
Specificity This antibody recognises beta and gamma actin in Human samples. It probably also recognises all the other known forms of Human actin. This antibody detects a single clean band in Human, Mouse, Rat, Chicken and Drosophila samples. In Xenopus laevis a secondary band is detected at about 30kDa. We are unsure whether this is cross-reaction with another actin isoform or merely non-specific. In Cow a doublet is detected, which probably represents different forms of actin.

Tested applications Suitable for: WB, IHC-P
Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse, Rat, Chicken, Cow, Human, Xenopus laevis, Drosophila melanogaster, Zebrafish
Predicted to work with: Rabbit, Saccharomyces cerevisiae, Orangutan

Immunogen Synthetic peptide corresponding to Human Actin aa 350 to the C-terminus conjugated to keyhole limpet haemocyanin. (Peptide available as ab13771)

Positive control HeLa whole cell lysate or mouse brain lysate. IHC-P - Human Colon FFPE tissue section

Properties

Form Liquid
Storage instructions 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.
Storage buffer pH: 7.40
Preservative: 0.02% Sodium azide
Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity Immunogen affinity purified
Clonality Polyclonal
Isotype

IgG

Applications

Our Abpromise guarantee covers the use of ab1801 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>WB</td>
<td></td>
<td>1/1000. Detects a band of approximately 42 kDa (predicted molecular weight: 42 kDa). Block in 5% BSA. Blocking in milk significantly reduces the signal.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
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</table>

Application notes

Is unsuitable for ICC/IF.

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 ACTA1 are the cause of nemaline myopathy type 3 (NEM3) [MIM:161800]. A form of nemaline myopathy. Nemaline myopathies are muscular disorders characterized by muscle weakness of varying severity and onset, and abnormal thread-or rod-like structures in muscle fibers on histologic examination. The phenotype at histological level is variable. Some patients present areas devoid of oxidative activity containing (cores) within myofibers. Core lesions are unstructured and poorly circumscribed.

Defects in ACTA1 are a cause of myopathy congenital with excess of thin myofilaments (MPCETM) [MIM:161800]. A congenital muscular disorder characterized at histological level by areas of sarcoplasm devoid of normal myofibrils and mitochondria, and replaced with dense masses of thin filaments. Central cores, rods, ragged red fibers, and necrosis are absent.

Defects in ACTA1 are a cause of congenital myopathy with fiber-type disproportion (CFTD) [MIM:255310]; also known as congenital fiber-type disproportion myopathy (CFTDM). CFTD is a genetically heterogeneous disorder in which there is relative hypotrophy of type 1 muscle fibers compared to type 2 fibers on skeletal muscle biopsy. However, these findings are not specific and can be found in many different myopathic and neuropathic conditions.

Sequence similarities

Belongs to the actin family.

Cellular localization

Cytoplasm > cytoskeleton.

Images
IHC image of ab1801 staining Actin in normal 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 ab1801, 5µl/ml 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 negative 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

**All lanes**: Anti-Actin antibody - Loading Control (ab1801) at 1 µg/ml

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

**Lane 2**: Brain (Rat) Tissue Lysate

**Lane 3**: Brain (Mouse) Tissue Lysate

**Lane 4**: NIH 3T3 whole cell lysate (ab7179)

Lysates/proteins at 10 µg per lane.

**Secondary**

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

Developed using the ECL technique.

Performed under reducing conditions.

**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 2% Bovine Serum Albumin before being incubated with ab1801 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406.

Western blot analysis of HeLa cells treated for 12 hours with hesperidin (h) (2.5 μg/ml, 4.01 μM), mangiferin (5 μg/ml, 11.84 μM) (m), and hesperidin (2.5 μg/ml, 4.01 μM) in a presence of mangiferin (5 μg/ml, 11.84 μM) (h+m). Immunoblotting was performed with the following primary antibodies: Bax (ab32503), BCL2 (ab59348), beta actin (ab1801), and caspase 8. After the washing steps, the membranes were incubated with goat anti-rabbit IgG (H+L) or with goat anti-mouse IgG (H+L) HRP-conjugated secondary antibodies and detected using ECL. Densitometry was performed using Image Lab software v. 4.1 (BioRad).

**Top panel:** Following 12h of treatment of HeLa cells with hesperidin (h), mangiferin (m), and hesperidin in a presence of mangiferin (h+m), the mRNA levels were monitored in real-time PCR experiments. The BAX and BCL2 mRNA levels results from 2 independent experiments (n=8) are plotted relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 18S rRNA levels and expressed as a fold change over the EtOH control. Error bars represent standard derivations.

**Bottom panel:** Following 12h of treatment of HeLa cells with hesperidin (h), mangiferin (m), and hesperidin in a presence of mangiferin (h+m), the protein levels of Bax and BCL2 were detected with SDS-PAGE and Western Blot and related to beta actin levels.
**All lanes**: Anti-Actin antibody - Loading Control (ab1801) at 1 µg/ml

**Lane 1**: Brain (Mouse) Tissue Lysate (ab27253)
**Lane 2**: NIH 3T3 (Mouse) Whole Cell Lysate (ab52956)

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 42 kDa

**Exposure time**: 30 seconds

**All lanes**: Anti-Actin antibody - Loading Control (ab1801) at 1/1000 dilution

**All lanes**: Whole cell lysates prepared from HUVEC cells

Lysates/proteins at 15 µg per lane.

**Secondary**

**All lanes**: HRP-conjugated goat polyclonal to rabbit Ig at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 42 kDa
Exposure time: 30 seconds

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