Product Datasheet

beta-Actin Antibody (AC-15)
NB600-501

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

Reviews: 19  Publications: 235

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB600-501

Updated 10/13/2016 v.20.1
## Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>This product is unpurified. The exact concentration of antibody is not quantifiable.</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at 4°C short term. Aliquot and store at -20°C long term. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Clone</td>
<td>AC-15</td>
</tr>
<tr>
<td>Preservative</td>
<td>15mM Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG1</td>
</tr>
<tr>
<td>Purity</td>
<td>Ascites</td>
</tr>
<tr>
<td>Buffer</td>
<td>Ascitic fluid</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
<td>42 kDa</td>
</tr>
</tbody>
</table>

## Product Description

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>Mouse</td>
</tr>
<tr>
<td>Gene ID</td>
<td>60</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>ACTB</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat, Porcine, Bovine, Canine, Chicken, Feline, Fish, Guinea Pig, Hamster, Leech, Mammal, Primate, Rabbit, Sheep, Squirrel, Zebrafish, Drosophila (Negative)</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>The antibody cross reacts with β-actin expressing cells in carp, leech tissues (Hirudo medicinalis), ground squirrel. Does not cross react with adult cardiac, skeletal muscle, drosophila or amoeba β actin. Hamster reactivity reported in scientific literature (PMID: 24478435). Mammal reactivity reported in scientific literature (PMID: 25130694) Please note that this antibody is reactive to Mouse and derived from the same host, Mouse. Additional Mouse on Mouse blocking steps may be required for IHC and ICC experiments. Please contact Technical Support for more information.</td>
</tr>
<tr>
<td>Specificity/Sensitivity</td>
<td>In staining of chicken gizzard ultrathin tissue cryosections, the antibody labels the dense bodies and longitudinal channels linking consecutive dense bodies that are also occupied by desmin and the membrane-associated dense plaque. It does not stain adult cardiac and skeletal muscles except for traces due to contaminations of the sample with non-muscle cells, or if embryonic tissue is being used. The epitope recognized by the antibody is resistant to formalin-fixed and paraffin-embedding.</td>
</tr>
<tr>
<td>Immunogen</td>
<td>slightly modified beta-cytoplasmic actin N-terminal peptide, Ac-Asp-Asp-Asp-Ile-Ala-Ala-Leu-Val-Ile-Asp-Asn-Gly-Ser-Gly-Lys, conjugated to KLH.</td>
</tr>
</tbody>
</table>

## Product Application Details

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applications</td>
<td>Western Blot, Simple Western, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation</td>
</tr>
</tbody>
</table>
### Recommended Dilutions

<table>
<thead>
<tr>
<th>Method</th>
<th>Dilution Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Blot</td>
<td>1:5000 - 1:10000</td>
</tr>
<tr>
<td>Simple Western</td>
<td>1:25</td>
</tr>
<tr>
<td>Flow Cytometry, ELISA</td>
<td>1:100 - 1:2000</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>1:10 - 1:500</td>
</tr>
<tr>
<td>Immunocytochemistry/Immunofluorescence</td>
<td>1:1000 - 1:2000</td>
</tr>
<tr>
<td>Immunoprecipitation</td>
<td>1:10 - 1:500</td>
</tr>
<tr>
<td>Immunohistochemistry-Paraffin</td>
<td>1:10 - 1:500</td>
</tr>
<tr>
<td>Immunohistochemistry-Frozen</td>
<td>1:500</td>
</tr>
</tbody>
</table>

### Application Notes

This antibody is useful for Western blot, ELISA, immunohistochemistry and Immunocytochemistry. The antibody can be used for staining of acetone-fixed frozen sections, EM preparations, and microinjection experiments. This antibody is resistant to formalin fixation and paraffin embedding. B5, methacarn, ethanol or Bouin’s solutions may also be used as fixatives. Use in immunoprecipitation reported in scientific literature (PMID 23647384). In Simple Western only 10-15 uL of the recommended dilution is used per data point. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

### Images

**Western Blot:** beta-Actin Antibody (AC-15) [NB600-501] - beta-Actin expression in human glioma cells using anti-beta-Actin antibody. Image from verified customer review.

**Immunocytochemistry/Immunofluorescence:** beta-Actin Antibody (AC-15) [NB600-501] - HS-68 cells.

**Immunohistochemistry:** beta-Actin Antibody (AC-15) [NB600-501] - Breast, Epithelium 40x.
Flow Cytometry: beta-Actin Antibody (AC-15) [NB600-501] - analysis of HeLa cells using mouse Monoclonal beta-Actin antibody (Orange) and Isotype control Antibody (Blue).

Western Blot: beta-Actin Antibody (AC-15) [NB600-501] - beta-Actin expression in CHO, GC-1, HeLa, Cos-7 and SH-SY5Y cell lysates using anti-beta-Actin antibody. The primary antibody was used at a dilution of 1:10,000 for 1 hour. Image from verified customer review.


Western Blot: beta-Actin Antibody (AC-15) [NB600-501] - beta-Actin expression in human cell lines (Caco-2, T84, HCT116, HT29, DU145, BPH1, HeLa). Image from verified customer review.
Western Blot: beta-Actin Antibody (AC-15) [NB600-501] - MCDK cells induced with doxycycline to control the expression of the gene of interest. Beta actin blocking confirms the albumin assay showing that an equal amount of lysate was loaded in each lane.

Western Blot: beta-Actin Antibody (AC-15) [NB600-501] - Whole cell extract of human fibroblasts was separated on SDS-PAGE and blotted with Monoclonal Anti-alpha-Actin. The antibody was developed with Goat Anti-Mouse IgG, Peroxidase conjugate and AEC substrate. Lanes A: Antibody dilution 1:5,000 B: Negative control (only secondary antibody).


Immunocytochemistry/Immunofluorescence: beta-Actin Antibody (AC-15) [NB600-501] - Double exposure micrographs of longitudinal, expanded (a,b) and transverse (c) semi-thin cryosections of chicken gizzard muscle double labeled with Monoclonal Anti-alpha-Actin and polyclonal antibodies to chicken gizzard myosin (red). d-f. Longitudinal, expanded cryosections of chicken gizzard muscle double labeled with polyclonal Anti-alpha-Actinin (green, d, e, red, f) in combination with antibodies to chicken gizzard myosin, antibodies to chicken desmin and Monoclonal Anti-Beta Actin, (f, green). Bars, 10 mm. From Dr. J. V. Small, Institute of Molecular Biology, Academy of Sciences, Salzburg.

Flow Cytometry: beta-Actin Antibody (AC-15) [NB600-501] - Analysis using the HRP conjugate of NB600-501. Electropherogram image(s) of corresponding Simple Western lane view. Beta-Actin antibody was used at 1:500 dilution on Hela, MCF-7, SH-SY5Y, & Jurkat lysate(s).

Simple Western: beta-Actin Antibody (AC-15) [NB600-501] - Analysis using the HRP conjugate of NB600-501. Simple Western lane view shows a specific band for Beta-Actin in 0.2 mg/ml of Hela, MCF-7, SH-SY5Y, & Jurkat lysate(s). This experiment was performed under reducing conditions using the 12-230kDa separation system. * Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.


Wu L, Zhang Z, Pan X, Wang Z. Expression and contribution of the HIF1a/VEGF signaling pathway to luteal development and function in pregnant rats Mol Med Rep (Rat)

Details:
This publication used the HRP conjugated form of this antibody (Cat# NB600-501H).


Details:
beta-Actin antibody used as a loading control for WB on human samples (protein lysates from differentiating cells – hLiver, Huh7.5.1 and HepG2 cells) (Fig. 2C).


Carmony KC. Elucidating Proteasome Catalytic Subunit Composition and Its Role in Proteasome Inhibitor Resistance. Thesis. Apr 18 2016 12:00AM (WB, Human)


Details:
Beta actin antibody was used for Western blot application in experiments involving human neuroblastoma cancer cell lines namely SK-N-AS, SK-NSH and NB1691.


More publications at http://www.novusbio.com/NB600-501
Procedures

Immunohistochemistry Protocol for Beta Actin Antibody (NB600-501)

IHC-FFPE sections:

I. Deyparaffinization:

A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

A. Place slides in peroxidase quenching solution: 15-30 minutes.
To Prepare 200 ml of Quenching Solution:
Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:

A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water: 2 changes for 2 minutes each.

IV. Immunostaining Procedure:

A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:
Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
All steps in which Xylene is used should be performed in a fume hood.
For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary. Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).
Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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info@bio-techne.com

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General: novus@novusbio.com

Products Related to NB600-501

<table>
<thead>
<tr>
<th>Product Code</th>
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<tbody>
<tr>
<td>HAF007</td>
<td>Goat anti-Mouse IgG Secondary Antibody [HRP (Horseradish Peroxidase)]</td>
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<tr>
<td>NB720-B</td>
<td>Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]</td>
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<tr>
<td>NBP1-97005</td>
<td>Mouse IgG1 Isotype Control</td>
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<tr>
<td>NB600-501H</td>
<td>beta-Actin Antibody (AC-15) [HRP]</td>
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