Product Datasheet

p62/SQSTM1 Antibody
NBP1-48320

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

Reviews: 7  Publications: 61

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Updated 1/7/2020 v.20.1

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### NBP1-48320  
**p62/SQSTM1 Antibody**

#### Product Information

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at -20C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.02% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS</td>
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</table>

#### Product Description

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Gene ID</td>
<td>8878</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>SQSTM1</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>Immunogen sequence is 100% identical to several non-human primates/monkey species. Immunogen sequence homology to other species: Chinese hamster (98%), Sheep (98%), Bovine (97%), Porcine (96%), Canine (91%), Daphnia magna, a cladoceran/crustaceans invertebrate (97%) and Duck and several other Birds (84%).</td>
</tr>
<tr>
<td>Immunogen</td>
<td>A genomic peptide made to c-t of the human p62/SQSTM1 protein (within residues 300-440). [Swiss-Prot Q13501]</td>
</tr>
<tr>
<td>Notes</td>
<td>Manufactured by Genomic Antibody Technology™. GAT FAQs</td>
</tr>
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</table>

#### Product Application Details

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Applications</td>
<td>Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin</td>
</tr>
<tr>
<td>Recommended Dilutions</td>
<td>Western Blot 1:4000, Simple Western 1:25, Flow Cytometry, Immunohistochemistry, Immunocytochemistry/Immunofluorescence 1:25-1:200, Immunohistochemistry-Paraffin, Immunohistochemistry-Frozen</td>
</tr>
<tr>
<td>Application Notes</td>
<td>Use of this antibody in IHC-Frozen is reported in (PMID: 25014022) and in IHC-paraffin is reported in (PMID: 27497324). Use in FLOW reported in scientific literature (PMID: 29258618). In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.</td>
</tr>
</tbody>
</table>
Western Blot: p62/SQSTM1 Antibody [NBP1-48320] - Cultured HeLa cells were treated with or without 50 uM chloroquine for 24 hours as indicated. Cell lysates were prepared and separated on a 12% gel by SDS-PAGE. Protein was transferred to PVDF membrane and blocked in 5% non-fat milk. The membrane was then probed with 1 ug/ml anti-p62/SQSTM1 in 1% milk and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Note the upregulation of p62 (arrowhead) in response to chloroquine treatment and the blockage of autophagy.

Immunocytochemistry/Immunofluorescence: p62/SQSTM1 Antibody [NBP1-48320] - HeLa cells were treated overnight with 50uM CQ, then fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-p62(SQSTM1) at a 1:200 dilution overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

Immunocytochemistry/Immunofluorescence: p62/SQSTM1 Antibody [NBP1-48320] - Untreated HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-p62(SQSTM1) at a 1:200 dilution overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

Simple Western: p62/SQSTM1 Antibody [NBP1-48320] - Lane view shows a specific band for p62/SQSTM1 in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.
Publications

Karuppan MKM, Ojha CR, Rodriguez M, Lapierre J. Zika virus infection during pregnancy and induced brain pathology in beclin1-deficient mouse model bioRxiv Nov 15 2019 12:00AM (WB, Mouse)


Song J, Zhao X, Feng Y et al. Involvement of proapoptotic genes in autophagic cell death induced by irradiation Cell Death Discov. 2017 Dec 03 [PMID: 31098300] (WB, Human)


la Fuente FP, Quezada L, SepUlveda C et al. Exercise regulates lipid droplet dynamics in normal and fatty liver Biochim Biophys Acta Mol Cell Biol Lipids Aug 29 2019 12:00AM [PMID: 31473346] (WB, Mouse)


Details:
Dilution for WB: 1:1000


Tedeschi V, Petrozziello T, Sisalli MJ et al. The activation of Mucolipin TRP channel 1 (TRPML1) protects motor neurons from L-BMAA neurotoxicity by promoting autophagic clearance Sci Rep Jul 24 2019 12:00AM [PMID: 31341250] (WB, Mouse)

Qiu Y, Yao J, Jia L et al. Shifting the balance of autophagy and proteasome activation reduces proteotoxic cell death: a novel therapeutic approach for restoring photoreceptor homeostasis Cell Death Dis Jul 18 2019 12:00AM [PMID: 31320609] (WB, Mouse)

More publications at http://www.novusbio.com/NBP1-48320
Procedures

Protocol specific for p62/SQSTM1 Antibody (NBP1-48320)

Materials

1X PBS
Sample buffer, 2X Laemmli buffer: 4% SDS, 5% 2-mercaptoethanol (BME), 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris HCl, pH 6.8
1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3
1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol Adjust to pH 8.3
TBS
TBST, TBS and 0.1% Tween
Blocking solution: TBST, 5% non-fat dry milk
rabbit anti-p62/SQSMT1 primary antibody (NBP1-48320) in blocking buffer (~2 mg/mL)

Methods

1. Grow cells (e.g. HeLa or Neuro2A) in vitro to semi-confluency (70-75%).
2. Rinse cells with ice-cold 1X PBS and lyse cells with sample buffer.
3. Sonicate and incubate cells for 5 minutes at 95°C.
   Tip: Cells are lysed directly in sample buffer.
   Note: For cell lysis, an SDS containing buffer is recommended to identify the entire cellular pool of p62/SQSTM1.
4. Load 10-40 mg/lane of sample on a 12% polyacrylamide gel (SDS-PAGE).
   Note: To determine autophagic flux based on p62/SQSTM, immunoblot analysis should include samples treated with autophagy inducers and inhibitors.
5. Transfer proteins to a Nitrocellulose membrane for 60 minutes at 100V.
   Tip: For more information on Western Blotting, see our Western Blot handbook: https://images.novusbio.com/design/BR_westernblotguide_042816b.pdf
6. After transfer, rinse the membrane with dH2O and stain with Ponceau S for 1-2 minutes to confirm efficiency of protein transfer.
7. Rinse the membrane in dH2O to remove excess stain and mark the loaded lanes and molecular weight markers using a pencil.
8. Block the membrane using blocking buffer solution (5% BSA in TBST) for 1 hour at room temperature.
9. Rinse the membrane with TBST for 5 minutes.
10. Dilute the rabbit anti-p62/SQSTM1 primary antibody (NBP1-48320) in blocking buffer (~2 mg/mL) and incubate the membrane for 1 hour at room temperature.
11. Rinse the membrane with dH2O.
12. Rinse the membrane with TBST, 3 times for 10 minutes each.
13. Incubate the membrane with diluted secondary antibody, according with product's specification, (e.g. anti-rabbit-IgG HRP-conjugated) in blocking buffer for 1 hour at room temperature.
   Note: Tween-20 may be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.
14. Rinse the membrane with TBST, 3 times for 10 minutes each.

15. Apply the detection reagent of choice (e.g. BioFX Super Plus ECL) in accordance with the manufacturer's instructions.
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.

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