Product Datasheet

Sodium Potassium ATPase Alpha 1 Antibody
NB300-146SS

Unit Size: 0.025 ml
Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

Reviews: 2  Publications: 59

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB300-146

Updated 6/15/2014 v.20.1
# Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.025 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>This product is unpurified. The exact concentration of antibody is not quantifiable.</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Aliquot and store at -20°C or -80°C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Monoclonal</td>
</tr>
<tr>
<td><strong>Clone</strong></td>
<td>464.6</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.1% Sodium Azide</td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
<td>IgG1 Kappa</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Ascites</td>
</tr>
<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>112 kDa</td>
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</tbody>
</table>

## Product Description

| **Host** | Mouse |
| **Gene ID** | 476 |
| **Gene Symbol** | ATP1A1 |
| **Species** | Human, Mouse, Rat, Bovine, Canine, Drosophila, Guinea Pig, Primate, Porcine, Rabbit, Sheep, Xenopus, Yeast |
| **Species Reactivity** | Human, bovine, porcine, monkey, murine, rabbit, guinea pig, canine, sheep, rat, Drosophila, Xenopus and Yeast. |
| **Marker** | Plasma Membrane Marker |
| **Specificity/Sensitivity** | This is specific for Na,K-ATPase alpha 1 subunit. |
| **Immunogen** | Purified Sodium Potassium ATPase Alpha 1 from rabbit renal outer medulla. [UniProt# Q9N0Z6] |

## Product Application Details

| **Applications** | Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin |
| **Recommended Dilutions** | Flow Cytometry 1:50-1:200, Immunocytochemistry/Immunofluorescence 1:50-1:1000, Immunohistochemistry 1:200, Immunohistochemistry-Paraffin 1:200, Western Blot 1:1000-1:10000 |
| **Application Notes** | This Sodium Potassium ATPase (464.6) antibody is useful for Immunohistochemistry on paraffin-embedded sections, Immunocytochemistry/Immunofluorescence, Flow Cytometry and Western Blot. By Western Blot, a distinct band at 112 kDa is seen. Do not boil the sample prior to loading on the gel for Western Blot (60 degrees Celsius appears to work fine). |
Images

Western Blot: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - Detection of Sodium Potassium ATPase Alpha 1 in human postmortem prefrontal cortex tissue. Photo courtesy of product review by verified customer.

Immunocytochemistry/Immunofluorescence: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - Immunocytochemistry: Detection of ATPA1 (Green) in HepG2 cells using NB300-146. Nuclei (Blue) were counterstained using Hoechst 33258.

Immunohistochemistry: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - Staining of endometrial glands within the uterus using NB300-146. Note the absence of staining in the surrounding myometrial smooth muscle.

Flow Cytometry: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - Detection of Sodium Potassium ATPase Alpha 1 in fixed Hela cells.
Western Blot: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - Analysis detecting Na, K-ATPase (alpha) in porcine proximal tubule protein.

**Publications**


More publications at [http://www.novusbio.com/NB300-146](http://www.novusbio.com/NB300-146)
Procedures

Protocol specific for Sodium Potassium ATPase Alpha 1 Antibody (NB300-146)

Procedure Guide for NB 300-146 Monoclonal anti-Na-K-ATPase Antibody (464.6)

I. Western Blot Procedure
1. Run ~20 ug of Triton-treated porcine proximal tubule extract protein on a 7.5% SDS-PAGE gel.*
2. Transfer protein to the membrane using a Tris-Glycine/Methanol buffer.
3. Block membrane with TBST/5% NFDM for 30 min. at room temperature (~23-27 degrees C).
4. Wash membrane twice, for 5 minutes each, with TBST.
5. Incubate membrane with 1:5,000 dilution of NB300-146 (anti-Na,K-ATPase), diluted in TBST, for 1 hour at room temperature.
6. Wash membrane once for 15 minutes, then four times for 5 minutes each, with TBST.
7. Incubate membrane with 1:15,000 dilution of goat anti-mouse IgG-HRP [(, diluted in TBST, for 1 hour at room temperature.
8. Wash membrane once for 15 minutes, then four times for 5 minutes each, with TBST.
9. Detect cross-reacting proteins using Chemiluminescent substrate according to manufacturers guidelines.* NOTE: Do not boil the protein samples, as boiling causes aggregation of the Na,K-ATPase. The aggregate band will appear at ~150 kDa on Western Blots.

II. Quench Endogenous Peroxidase: IHC-FFPE sections

1. Deparaffinization:
   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.

2. 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.

3. 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.

4. 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 Celsius 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 guarantee, please visit www.novusbio.com/guarantee.