

#4020 Store at  $-20^{\circ}\text{C}$

# Phospho-Na,K-ATPase $\alpha 1$ (Ser16) Antibody

- Small 100  $\mu\text{l}$   
(10 Western mini-blots)
- Petite 40  $\mu\text{l}$   
(4 Western mini-blots)



**Orders** ■ 877-616-CELL (2355)  
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This product is for *in vitro* research use only and is not intended for use in humans or animals.

| Applications    | Species Cross-Reactivity* | Molecular Wt. | Source |
|-----------------|---------------------------|---------------|--------|
| W<br>Endogenous | R, (M, B)                 | 100 kDa       | Rabbit |

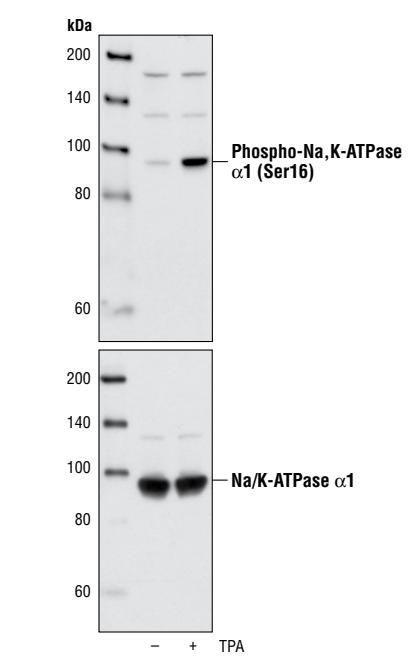
**Background:** The Na,K-ATPase is an integral membrane heterodimer belonging to the P-type ATPase family. This ion channel uses the energy derived from ATP hydrolysis to maintain membrane potential by driving sodium export and potassium import across the plasma membrane against their electrochemical gradients. It is composed of a catalytic  $\alpha$  subunit and a  $\beta$  subunit (reviewed in 1). Several phosphorylation sites have been identified for the  $\alpha 1$  subunit. Tyr10 is phosphorylated by an as yet undetermined kinase (2), Ser16 and Ser23 are phosphorylated by PKC, and Ser943 is phosphorylated by PKA (3-5). All of these sites have been implicated in the regulation of enzyme activity in response to hormones and neurotransmitters, altering trafficking and kinetic properties of Na,K-ATPase. Altered phosphorylation in response to angiotensin II stimulates activity in rat proximal tubule (6). Na,K-ATPase is also involved in other signal transduction pathways. Insulin regulates its localization in differentiated primary human skeletal muscle cells, and this regulation is dependent on ERK1/2 phosphorylation of the  $\alpha$  subunit (7). Na,K-ATPase and Src form a signaling receptor complex that affects regulation of Src kinase activity and, subsequently, its downstream effectors (8,9).

**Specificity/Sensitivity:** Phospho-Na,K-ATPase  $\alpha 1$  (Ser16) Antibody recognizes endogenous levels of Na,K-ATPase  $\alpha 1$  only when phosphorylated at Ser16. The residue number, Ser16, is based on the sequence of the immature form of the protein, corresponding to Ser11 of the mature cleaved form.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser16 of rat Na,K-ATPase  $\alpha 1$ . Antibodies are purified using protein A and peptide affinity chromatography.

**Background References:**

- (1) Therien, A.G. and Blostein, R. (2000) *Am. J. Physiol. Cell Physiol.* 279, C541-566.
- (2) Féraille, E. et al. (1999) *Mol. Biol. Cell* 10, 2847-2859.
- (3) Fisone, G. et al. (1994) *J. Biol. Chem.* 269, 9368-9373.
- (4) Feschenko, M.S. and Sweadner, K.J. (1995) *J. Biol. Chem.* 270, 14072-14077.



Western blot analysis of extracts from PC-12 cells, untreated or TPA-treated, using Phospho-Na,K-ATPase  $\alpha 1$  (Ser16) Antibody (upper) or total Na/K ATPase  $\alpha 1$  Antibody #3010 (lower).

- (5) Beguin, P. et al. (1994) *J. Biol. Chem.* 269, 24437-24445.
- (6) Yingst, D.R. et al. (2004) *Am. J. Physiol. Renal Physiol.* 287, F713-F721.
- (7) Al-Khalili, L. et al. (2004) *J. Biol. Chem.* 279, 25211-25218.
- (8) Tian, J. et al. (2006) *Mol. Biol. Cell* 17, 317-326.
- (9) Liang, M. et al. (2006) *J. Biol. Chem.* 281, 19709-19719.

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu\text{g}/\text{ml}$  BSA and 50% glycerol. Store at  $-20^{\circ}\text{C}$ . Do not aliquot the antibody.

\*Species cross-reactivity is determined by Western blot.

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

- Companion Products:**
- Phospho-Na,K-ATPase  $\alpha 1$  (Ser23) Antibody #4006
  - Na,K-ATPase  $\alpha 1$  Antibody #3010
  - Phototope<sup>®</sup>-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
  - Anti-rabbit IgG, HRP-linked Antibody #7074
  - Prestained Protein Marker, Broad Range (Premixed Format) #7720
  - Biotinylated Protein Ladder Detection Pack #7727
  - 20X LumiGLO<sup>®</sup> Reagent and 20X Peroxide #7003

## Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.