

## Certificate of Analysis

### Anti-phospho-AMPK $\alpha$ (Thr172)

(rabbit immunoaffinity purified IgG)

Catalog # 07-626

Lot # 30851

**Immunogen:** KLH-conjugated, synthetic peptide corresponding to amino acids surrounding phosphorylated Thr172 of human AMP-activated protein kinase alpha 2 (AMPK $\alpha$ 2). The immunogenic site is conserved in AMPK $\alpha$ 1, isoform 1 (Thr174), AMPK $\alpha$ 1, isoform 2 (Thr189), and AMPK $\alpha$ 2 (Thr172). Accession number NM\_006251. The immunizing sequence is identical in rat.

**Specificity:** Recognizes phospho-AMPK $\alpha$  (Thr172), Mr 63kDa. A non-specific protein was also detected, Mr 160kDa.

**Species Cross-reactivity:** Human. Predicted to cross-react with rat based on sequence homology.

**Formulation:** 200 $\mu$ l of immunoaffinity purified rabbit IgG in 70% storage buffer (0.2M Tris-glycine, pH 7.4, 0.15M NaCl, 7.5mg/ml BSA and 0.05% sodium azide) and 30% glycerol. Store at -20°C.

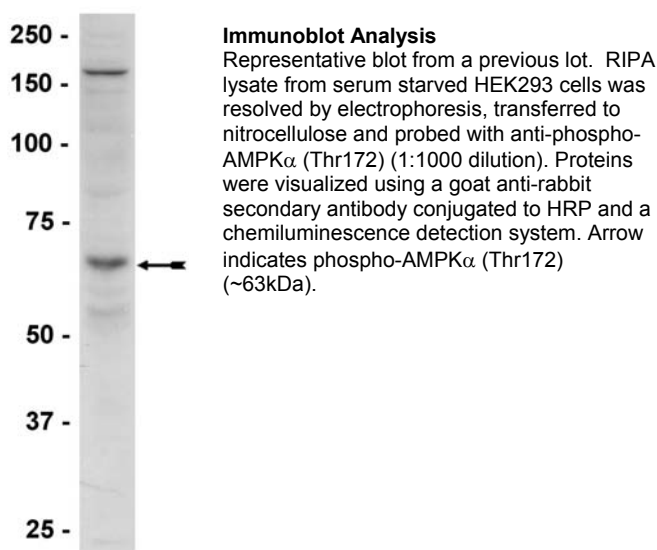
**Storage and Stability:** Stable for 2 years at -20°C from date of shipment.

**Handling Recommendations:** Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

### Quality Control Testing

**Immunoblot Analysis:** 1:500-1:1000 dilutions of this lot detected phospho-AMPK $\alpha$  (Thr172) in RIPA lysates from serum starved HEK293 cells.



### General References:

1. Kemp, B.E., *et al.*, *TIBS* **24**: 22-25, 1999.
2. Michell, B.J., *et al.*, *J. Biol. Chem.* **271**: 28445-28450, 1996.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>, 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween<sup>®</sup>-20 (TBST-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with a **1:500-1:1000 dilution of anti-phospho-AMPK $\alpha$  (Thr172)**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBST-MLK for 1.5 hours with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween<sup>®</sup>-20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).