

Certificate of Analysis

Anti-phospho-Acetyl CoA Carboxylase (Ser79)

(rabbit polyclonal IgG)

Catalog # 07-303

Lot # 0701050669

Immunogen: KLH conjugated synthetic peptide (C-HM RSSM[pS]GLHLVK) corresponding to amino acid 73-85 of rat Acetyl CoA Carboxylase.

Specificity: Recognizes Acetyl CoA Carboxylase phosphorylated at Serine 79, Mr 257kDa.

Species Cross-reactivity: Mouse, rat, human, and rabbit.

Formulation: 200µg of protein A purified IgG in 200µl of 70% storage buffer (0.014M phosphate buffer, pH 7.6, 0.175M NaCl, 0.07% sodium azide) and 30% glycerol. Store at -20°C.

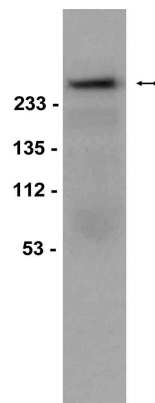
Storage and Stability: Stable for 2 years at -20°C from date of shipment.

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: 0.5-2µg/ml of this lot detected phosphorylated Acetyl CoA Carboxylase in RIPA lysates from mouse heart cytosol. Pretreating the blot with lambda phosphatase abolished antibody binding. A previous lot of this antibody was pretreated with the phospho-peptide immunogen, which also blocked antibody binding.



Immunoblot Analysis

Representative blot from a previous lot. Mouse heart cytosol cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Acetyl CoA Carboxylase (Ser79), (2µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Acetyl CoA Carboxylase (~257kDa).

Application References:

1. Andersson, U., *et al.*, *J. Biol. Chem.* **279**: 12005-8, 2004.
2. Chan, A. Y., *et al.*, *J. Biol. Chem.* **279**: 32771-9, 2004.
3. Miyazaki, M., *et al.*, *J. Biol. Chem.* **279**: 35017-24, 2004.

General References:

4. Kemp, B.E., *et al.*, *Trends Biochem Sci.* **24**: 22-25, 1999.
5. 2. Chen, Z.P., *et al.*, *Am. J. Physiol. Endocrinol. Metab.* **279**: E1202-E1206, 2000.

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 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 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), (TBS-MLK) for 2 hours at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-phospho-Acetyl CoA Carboxylase (Ser79)** diluted in freshly prepared TBS-MLK containing 0.05% Tween-20, overnight at 4 $^{\circ}$ C.
4. Wash the nitrocellulose three times 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 TBS-MLK containing 0.05% Tween-20, for 1.5 hours at room temperature with agitation.
6. Rinse the nitrocellulose with water twice
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
8. Wash the nitrocellulose with several changes of water for 1-2 hours.
9. Use detection method of choice (enhanced chemiluminescence was used).