

Certificate of Analysis

Anti-phospho-Adducin (Ser662)

(rabbit immunoaffinity purified IgG)

Catalog # 06-820

Lot # 0702051814

Immunogen: KLH-conjugated, synthetic peptide, (C-KKFRTP[pS]FLKKNK), corresponding to amino acids 656-668 of human γ -Adducin.

Specificity: Specific for phosphorylated serine 662 of γ -Adducin, Mr 80kDa. This lot of antibody also detects phosphorylated serine 724 of α -Adducin, Mr 120 kDa and phosphorylated serine 713 of β -Adducin, Mr 110 kDa. Both α and β -Adducin have 12/13 identity with the immunogenic motif.

Species Cross-reactivity: Human, dog, mouse and rat.

Formulation: 100 μ g of immunoaffinity purified rabbit IgG in 100 μ L of 0.014 M phosphate buffer, pH 7.6, 0.175 M NaCl, 0.07% sodium azide, and 30% glycerol. Liquid at -20°C.

Storage and Stability: Stable for 1 year at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

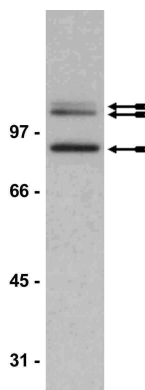
Immunoblot Analysis: 0.5-2 μ g/mL of this lot detected phosphorylated γ -Adducin, α -Adducin and β -Adducin in RIPA lysates from 3T3/NIH cells. Previous lots detected the Adducin isoforms in RIPA lysates from canine MDCK cells.

λ -phosphatase treatment:

Overnight λ -phosphatase treatment of 3T3/NIH cell lysate nitrocellulose blots abolished the detection of the phospho-Adducin isoforms.

Additional Research Applications

Immunohistochemistry: Not recommended.



Immunoblot Analysis

3T3/NIH cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Adducin (Ser662) (2 μ g/mL). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrows indicate phosphorylated γ -Adducin (80 kDa), α -Adducin (120 kDa) and β -Adducin (110 kDa).

General References:

1. Fowler, L., *et al.* (1998). *Cell Growth and Differ.* **9**:177-184,
2. Dong, L., *et al.* (1995). *J. Biol. Chem.* **270**:25534-25540,

Immunoblot Analysis with λ -Phosphatase treatment

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 EGTA; 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. Incubate nitrocellulose membranes for 1 hour in TBS (Tris-HCl, pH 7.4, 150mM NaCl) containing 1% bovine serum albumin (BSA) and 0.1% Triton X-100.
3. Incubate the membranes overnight in TBS with 1% BSA, 0.1% Triton X-100, 2mM MnCl₂. Add 400 Units/ml of λ -phosphatase (commercial source) to nitrocellulose membranes as needed.
4. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween-20 (TBST-MLK) for 30-40 minutes at room temperature with constant agitation.
5. Incubate the nitrocellulose with **0.5-2 μ g/mL of anti-phospho-Adducin (Ser662)**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
6. Wash the nitrocellulose twice with water.
7. 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 at room temperature with agitation.
8. Wash the nitrocellulose with water twice.
9. Wash the nitrocellulose in TBS-0.1% Tween 20 for 3-5 minutes.
10. Wash the nitrocellulose for 5-10 minutes each with 3-4 changes of water.
11. Use detection method of choice (enhanced chemiluminescence was used).