



cell signaling solutions

Certificate of Analysis

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Anti-phospho-Adducin (Ser662)

(rabbit immunoaffinity purified IgG)

Catalog # 06-820

Lot # 23970

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 120kDa and phosphorylated serine 713 of β -Adducin, Mr 110kDa. 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 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. 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

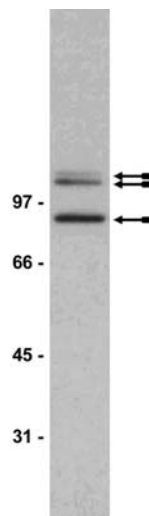
Immunoblot Analysis: 0.5-2 μ g/ml of this lot detected phosphorylated γ -Adducin, α -Adducin and β -Adducin in RIPA lysates from 3T3/NIH cells. Previous lots detected the Adducin isoforms in RIPA lysates from canine MDCK cells.

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 (80kDa), α -Adducin (120kDa) and β -Adducin (110 kDa).

General References:

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

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).