

## Anti-phospho-Adducin (Ser 662)

(rabbit immunoaffinity purified IgG)

Catalog # 06-820

Lot # 17560

**Immunogen:** KLH-conjugated, synthetic peptide, (C-KKFRTTP[ $\rho$ S]FLKKNK), corresponding to amino acids 656-668 of human  $\gamma$ -Adducin.

**Specificity:** Specific for phosphorylated serine 662 of  $\gamma$ -Adducin. This lot of antibody also detects phosphorylated serine 724 of  $\alpha$ -Adducin and phosphorylated serine 713 of  $\beta$ -Adducin. Both  $\alpha$  and  $\beta$ -Adducin have 12/13 identity with the immunogenic motif.

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

**Storage and Stability:** Stable for 1 year at  $-20^{\circ}\text{C}$  from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

**Formulation:** 100mg of immunoaffinity purified rabbit IgG in 100ml of 0.07M Tris-glycine, 0.105M NaCl, 0.035% sodium azide containing 30% glycerol. Liquid at  $-20^{\circ}\text{C}$ .

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS

### Quality Control Testing

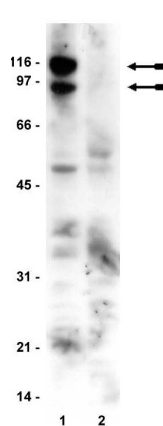
**Immunoblot Analysis:** 1 $\mu\text{g/ml}$  of this lot detected phosphorylated  $\alpha$ -Adducin (Mr ~120kDa) and  $\gamma$ -Adducin (Mr ~80kDa) in RIPA lysates from canine MDCK cells.

#### $\lambda$ -phosphatase treatment:

Overnight  $\lambda$ -phosphatase treatment of MDCK cell lysate nitrocellulose blots abolished the detection of phospho- $\alpha$  and  $\gamma$ -Adducin proteins.

### Additional Research Applications

**Immunohistochemistry:** Not recommended.



#### Immunoblot Analysis

MDCK cell lysate was resolved by electrophoresis, and transferred to nitrocellulose. Lane 1 of the blot remained untreated. Lane 2 was treated overnight with  $\lambda$ -phosphatase. Both lanes were subsequently incubated with anti-phospho-Adducin (Ser 662) (1  $\mu\text{g/ml}$ ). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrows indicate phosphorylated  $\alpha$ -Adducin (120kDa) and  $\gamma$ -Adducin (80 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 $\lambda$ -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 $\mu$ 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. 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 MnCl<sub>2</sub>. Add 400 Units/ml of  $\lambda$ -phosphatase (commercial source) to nitrocellulose membranes as needed.
4. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk containing 0.05% Tween-20 (TBST-MLK) for 40 minutes at 20-25°C with constant agitation.
5. Incubate the nitrocellulose with **1mg/ml of anti-phospho-Adducin (Ser 662)**, 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, 1:3000 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-1.0% 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).