



cell signaling solutions

## Certificate of Analysis

10 Old Barn Road • Lake Placid, NY 12946  
Technical Support: T: 800 548-7853 • F: 518 523-4513  
email: techserv@upstate.com  
Sales Department: T: 800 233-3991 • F: 781 890-7738  
Licensing Dept.: 800 310-4659  
www.upstate.com

### Anti-Cyclophilin A

(rabbit antiserum)

Catalog # 07-313

Lot # 24003

**Immunogen:** Recombinant full length protein corresponding to human Cyclophilin A.

**Specificity:** Recognizes Cyclophilin A, Mr 18kDa. Additional bands may be detected at higher concentrations of the antibody.

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

**Formulation:** 200 $\mu$ l of rabbit antiserum diluted in 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 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

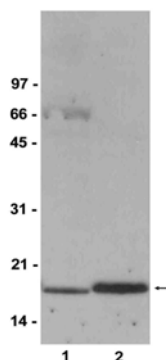
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Immunoblot Analysis:** 1:1000-1:10,000 dilution of this lot detected Cyclophilin A in RIPA lysates from non-stimulated A431 and rat lung cells.

**Included Positive Antigen Control:** Catalog # 12-301, non-stimulated A431 lysate. Add 2.5 $\mu$ l of 2-mercaptoethanol/100 $\mu$ l of lysate and boil for 5 minutes to reduce the preparation. Load 20 $\mu$ g of reduced lysate per lane for minigels.

### Additional Research Applications



#### Immunoblot Analysis

Representative blot from a previous lot. 20 $\mu$ g of rat lung (lane 1) and A431 cell lysate (lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Cyclophilin A (1:10,000 dilution). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Cyclophilin A (18kDa).

### General References:

1. Lee, S., *et al.*, *J. Biol. Chem.* **276**: 29826-29832, 2001.
2. Haendler, B., *et al.*, *EMBO J.* **6**: 947-950, 1987.
3. Pfuegel, G., *et al.*, *Nature* **361**: 91-94, 1993.

### 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 EGTA; 1mM PMSF; 1µg/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 with 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween 20 (TBST-MLK) for 30 minutes at room temperature with constant agitation.
3. Wash the nitrocellulose 2 x 5 minutes with water.
4. Incubate the nitrocellulose with **1:1000-1:10,000 dilution of anti-Cyclophilin A**, diluted in freshly prepared (TBST-MLK) overnight with agitation at 4°C.
5. Wash the nitrocellulose 3 x 10 minutes with water.
6. 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.
7. Wash the nitrocellulose 2 x 5 minutes with water.
8. Wash the nitrocellulose in TBS-0.05% Tween 20 for 15 minutes.
9. Rinse the nitrocellulose 3 x 10 minutes with water.
10. Use detection method of choice (enhanced chemiluminescence was used).