

# Anti-Cyclophilin A

Polyclonal Antibody

Cat. # 07-313

Lot # 31643

pack size: 200 µL

Store at -20°C

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS



## Certificate of Analysis

page 1 of 2

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB	H, M, R	IgG	N/A	Rb	~18 kDa	NP_066953

### Background

Immunophilins are a family of soluble cytosolic receptors capable of binding to one of two major immunosuppressant agents - cyclosporin A (CsA) or FK506. Proteins that bind FK506 are termed FK506 Binding Proteins (FKBPs) and those that bind cyclosporin A are called cyclophilins (CyP).

Both CyP:CsA and FKBP:FK506 complexes have been shown to inhibit calcineurin, a calcium and calmodulin dependent protein phosphatase which has been implicated as an important signaling enzyme in T-cell activation, providing a possible mechanism of immunosuppression by CsA and FK506.

Within the cyclophilin family, there are several different proteins which show a high degree of homology including CyPA, CyPB and CyPC. CyPA, also termed CyP-18, is the most abundant and ubiquitous cyclophilin found in all vertebrate tissues and is present in T-cells.

### Presentation

Rabbit polyclonal antiserum IgG in buffer containing 70% storage buffer 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide and 30% glycerol.

### Specificity

Recognizes Cyclophilin A, MW ~18 kDa. Additional bands may be detected at higher concentrations of the antibody.

### Species Cross-reactivity

Human, mouse and rat.

### Immunogen

Recombinant full length protein corresponding to human Cyclophilin A.

### Molecular Weight

~18 kDa

### Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon first thaw, 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.

### Control

Positive Control Included: Non-Stim A431 Cell Lysate (12-301).

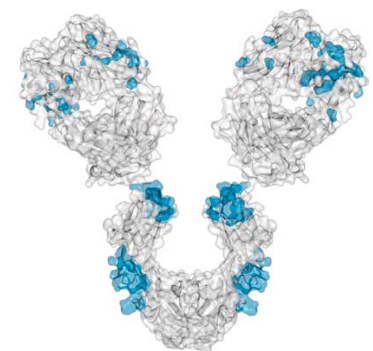
Other controls: Rat lung and rabbit liver cells.

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

### Quality Control Testing

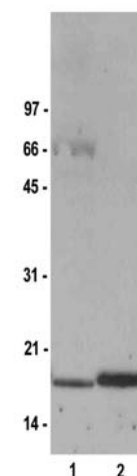
Evaluated by western blot on RIPA lysates from non-stimulated A431 and rat lung cells.

Western Blot Analysis: 1:1000-1:10,000 dilution of this antibody detected Cyclophilin A in RIPA lysates from non-stimulated A431 cells. A previous lot detected Cyclophilin A in RIPA lysates from rat lung cells.



### References

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



### Western Blot Analysis

Representative lot data. 20 µg 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 (~18 kDa).

**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence  
IH Immunohistochemistry (Tissue)

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

**Please visit [www.millipore.com](http://www.millipore.com) for additional product information, test data and references.**

Submit your published journal article, and earn credit toward future Millipore purchases. Visit [www.millipore.com/publicationrewards](http://www.millipore.com/publicationrewards) to learn more!

upstate | CHEMICON | Linco

THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO®  
IS NOW A PART OF MILLIPORE

**PROTOCOL**

**Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM 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) 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, 1:5000 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).

**RELATED PRODUCTS (specific)**

cat #	description
07-313	■ Anti-Cyclophilin A
M-004979	■ Cyclophilin A (human) SMARTpool® siRNA reagent
M-040767	■ Cyclophilin A (mouse) SMARTpool® siRNA reagent
61-003	■ Cyclophilin A siRNA/siAb™ Starter Kit (human)
61-004	■ Cyclophilin A siRNA/siAb™ Starter Kit (mouse)
62-079	■ siRNA plasmid, pKD-Cyclophilin A-v1
62-080	■ siRNA plasmid, pKD-Cyclophilin A-v2

**RELATED PRODUCTS (non-specific)**

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit [www.millipore.com](http://www.millipore.com) for additional product information, test data and references

28820 Single Oak Drive • Temecula, CA 92590

Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502

**FOR RESEARCH USE ONLY.** Not for use in diagnostic or therapeutic applications. Not for human or animal consumption. Purchase of this Product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited without prior written authorization from an authorized officer of Millipore Corporation.

Upstate®, Chemicon®, Linco® and all other trademarks are owned by Millipore Corporation. Copyright ©2008-2009 Millipore Corporation. All rights reserved.



We Buy 100% Certified Renewable Energy