



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

### Rabbit Anti-Chicken IgY, HRP Conjugate

Catalog # 12-341

Lot # 26668

**Immunogen:** Chicken IgY whole molecule.

**Specificity:** Immunoelectrophoresis resulted in a single precipitin arc against anti-peroxidase, anti-rabbit serum, chicken IgY, and chicken serum.

**Formulation:** 500µg of immunoaffinity purified rabbit IgG conjugated to horseradish peroxidase lyophilized from 0.02M Potassium Phosphate, 0.15M NaCl, pH 7.2, 10mg/ml BSA, and 0.01% gentamicin sulfate.

**Rehydration:** Add 500µl sterile, distilled water containing 50% glycerol, to make a 1mg/ml stock solution. Aliquot to avoid repeated freezing and thawing.

**Storage and Stability:** Stable for 2 years at 4°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product centrifuge the original vial after thawing and prior to removing the cap.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

#### Quality Control Testing

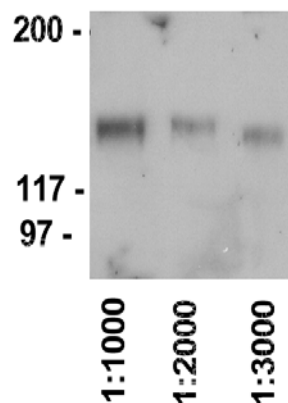
**Immunoblot Analysis:** A 1:1000-1:4000 dilution of previous lots appeared optimal for detecting the heavy and light chain of chicken IgY. A 1:1000 dilution of a previous lot appeared optimal for detecting IGF-1 receptors by immunoblotting with a chicken anti-IGF-1 antibody. Results may vary depending on antibody and/or cell/tissue lysate used. Use of sodium azide as a preservative will inhibit horseradish peroxidase enzyme activity.

**Note: Dilute just prior to use.**

#### Additional Research Applications

Also suitable for immunohistochemical, ELISA and other peroxidase-antibody assays.

Optimal concentrations for these applications need to be experimentally determined by the researcher.



#### Immunoblot Analysis

Representative blot from a previous lot. A 3T3/A31 RIPA cell lysate over-expressing the IGF-1 receptor was resolved by electrophoresis, transferred to nitrocellulose and probed with 1µg/ml chicken anti-IGF-1 receptor (Catalog # 06-429). Rabbit anti-Chicken IgY, HRP conjugate was used at the indicated dilutions. Proteins were visualized using a chemiluminescence detection system.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 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. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with primary antibody of choice diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in a **1:1000-1:4000 dilution of Rabbit anti-Chicken IgY, HRP Conjugate** secondary in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).