



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 # 22545

Immunogen: Chicken IgY whole molecule.

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

Storage and Stability: Stable for 1 year at -20°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. Also centrifuge if not completely clear after standing at room temperature.

Formulation: 500µg of immunoaffinity purified rabbit IgG conjugated to horseradish peroxidase in 500µl of 0.02M potassium phosphate, 0.015M NaCl, pH 7.2, 10mg/ml BSA, 30% glycerol and 0.01% gentamicin sulfate. Liquid.

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

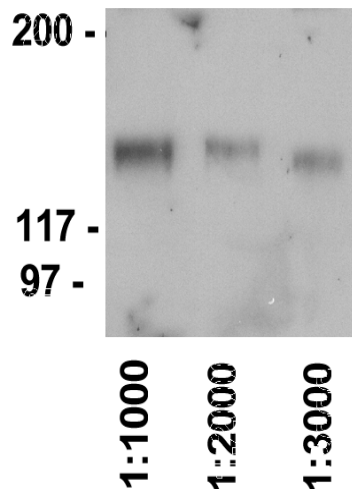
Quality Control Testing

Immunoblot Analysis: A 1:2000-1:4000 dilution of this lot 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.

Note: Only dilute prior to immediate 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 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 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. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in PBS (PBS-MLK) for 20 minutes at 20-25°C 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 **1:2000-1:4000 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).