
Certificate of Analysis

Anti-Mouse IgG, HRP-conjugate (goat polyclonal IgG) Catalog # 12-349 Lot # 20712

Immunogen: Highly purified whole mouse IgG.

Specificity: Recognizes mouse IgG, both heavy and light chains.

Species Cross-reactivity: Mouse.

Formulation: 500µg of goat IgG, conjugated with horseradish peroxidase, in 250µl 0.02M potassium phosphate, 0.15M NaCl, pH 7.2, with 10mg/ml BSA, 50% glycerol and 0.01% (w/v) gentamicin sulfate. Liquid at -20°C.

Storage and Stability: Stable for 1 year at -20°C from date of shipment.

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

Quality Control Testing

Immunoblot Analysis: This lot of antibody was tested at 1:1000, 1:3000, 1:4000 and 1:5000 dilutions in conjunction with either anti-phosphotyrosine antibody (Catalog # 05-321) or anti-Src antibody (Catalog # 05-184). This antibody detected phosphorylated EGF receptor and Src, respectively, in an EGF-stimulated A431 cell lysate

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; 1µg/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 PBS containing 3% nonfat dry milk (PBS-MLK) for 90 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **mouse primary antibody**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in **1:1000-1:5000 Anti-Mouse IgG, HRP-conjugate** in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with 4-5 changes of water.
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).