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

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Certificate of Analysis

Anti-Phosphotyrosine, recombinant 4G10

(mouse monoclonal $IgG_{2b\kappa}$)
Catalog # 05-777
Lot # 28782

Product Description: Produced from CHO cells expressing the 4G10 antibody heavy and light chain cDNAs. Heavy chain C-terminus has a hexa-histidine tag for purification and immobilization via Nickel affinity matrices. Patent pending.

Immunogen: Phosphotyramine-KLH.

Purity: >95% as determined by SDS-PAGE. Purified under neutral pH conditions by nickel affinity chromatography, eluted with 200mM imidazole.

Sterility: Filtered through a 0.2μ membrane and packaged aseptically.

Formulation: **1mg** of recombinant 4G10 mouse $IgG_{2b\kappa}$ in **1ml** of PBS, pH 7.5. Liquid at 4°C.

Storage and Stability: Stable for 2 years at 4°C from date of shipment. For maximum recovery of the product, centrifuge the original vial prior to removing the cap.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 0.5-2μg/ml of this lot detected tyrosine-phosphorylated proteins in a modified RIPA lysate from EGF-treated human A431 carcinoma cells.[1,2,3.]

Included Positive Antigen Control: Catalog # 12-302, EGF-stimulated A431 cell lysate is provided as a free positive antigen control for western immunoblotting. Aliquot as desired, refreeze immediately, and store at -20°C. The lysate is stable for 6 months at -20°C. Before use, **add 2.5μl of 2-mercaptoethanol/100μl of lysate and boil for 5 minutes to reduce the preparation.** Load 20μg of reduced lysate per lane for immunoblot analysis.

Immunoprecipitation: 2-4μg of this lot can immunoprecipitate quantitatively phosphotyrosine-containing proteins in the lysate of a confluent culture (10cm dish) of cells expressing an activated tyrosine kinase. To preserve phosphotyrosine, add 0.2mM sodium ortho-vanadate to the lysis buffer.

Immunoblot Analysis
Representative blot from a previous lot. EGF-stimulated A431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phosphotyrosine (1μg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

References:
1. Cohen, B., *et al*., Proc. Natl. Acad. Sci. USA. **87**: 4458-4462, 1990.
2. Druker, B.J., *et al*., New Eng. J. Med. **321:** 1383-1391, 1989.
3. Kanakura, Y., *et al*., J. Biol. Chem. **266:** 490-495, 1991.

Immunoprecipitation Protocol

1. Before beginning the immunoprecipitation, dilute the cell lysate to roughly 1μg/μl total cell protein in a microcentrifuge tube with PBS.
2. Add **2-4μg of anti-Phosphotyrosine, recombinant 4G10,** to 500μg-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100μl (50μl packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 70μl 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

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 EDTA; 1mM PMSF; 1μg/ml aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 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 **0.5-2μg/ml of anti-Phosphotyrosine, recombinant 4G10,** diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:5000 dilution, was used) 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 with a 10-30 second exposure was used).