

Certificate of Analysis

Anti-Phosphotyrosine, clone 4G10[®]
(mouse monoclonal IgG_{2bκ})
Catalog # 05-321
Lot # 0703053574

Immunogen: Phosphotyramine-KLH.

Antibody Class: IgG_{2bκ} mouse monoclonal antibody produced *in vitro* by mouse-mouse hybridoma 4G10[®] (FOX-NY [NS-1 derivative] myeloma x spleen cells). Purified by Protein G-Sepharose chromatography.

Formulation: 100 µg of protein G purified mouse monoclonal IgG_{2bκ} in 100 µL of 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide. Liquid at 4°C.

Storage and Stability: Stable for 2 years at 4°C from date of shipment. **NOTE: DO NOT FREEZE.** For maximum recovery of the product, centrifuge the original vial prior to removing the cap. If the product has accidentally been frozen and thawed, spin it at 13,000 x g for 10 minutes at 4°C. Save the supernatant for application.

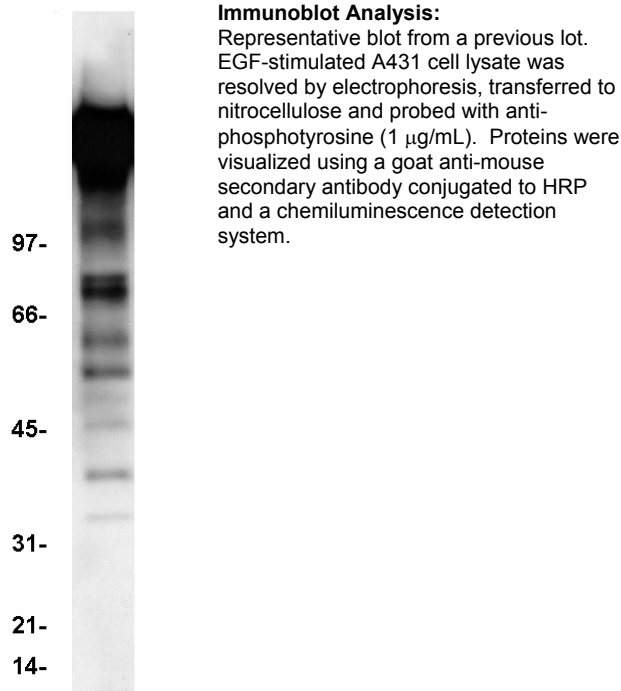
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.5-2 µg/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 2.5 µL of 2-mercaptoethanol per 100µL of lysate and boil for 5 minutes to reduce the preparation.** Load 20 µg of reduced lysate per lane for immunoblot analysis.

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



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. Add **2-4 µg of anti-Phosphotyrosine, clone 4G10[®]** and 60 µL (30 µL packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266) to 500 µL of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 hour.
3. 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.
4. Dilute the cell lysate to roughly 1 µg/µL total cell protein with PBS.
5. Add 500 µg-1 mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1 hour.
7. 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.
8. Resuspend the agarose beads in 60 µL 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. 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 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 3% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 45-90 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 µg/mL of anti-Phosphotyrosine, clone 4G10[®]** diluted in freshly prepared TBS-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, Catalog # 12-349, 1:4000 dilution, was used) in TBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBS-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 30 second exposure was used).