

## Anti-Phosphotyrosine

(mouse monoclonal IgG<sub>2bκ</sub>)

Catalog # 05-321

Lot # 18182

**Immunogen:** Phosphotyramine-KLH.

**Antibody Class:** IgG<sub>2bκ</sub> mouse monoclonal antibody produced *in vitro* by mouse-mouse hybridoma 4G10 (FOX-NY [NS-1 derivative] myeloma x spleen cells). Purified by Protein A-Sepharose chromatography.

**Formulation:** 100µg of mouse monoclonal IgG<sub>2bκ</sub> in 100µl of 0.01M Tris-HCl, pH 8.0, 0.15M NaCl, 0.02% sodium azide containing 10% glycerol. Protein was determined by a Bradford microtiter protein assay. Liquid.

**Storage and Stability:** Stable for 6 months 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.

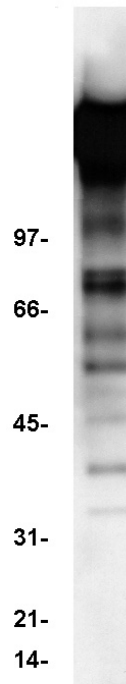
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### 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.<sup>1,2,3.</sup>

**Included Positive Antigen Control:** Catalog #12-302, EGF-stimulated A431 cell lysate is provided as a free positive antigen control for western immunoblotting with this antibody. Aliquot as desired, refreeze immediately, and store at -20°C. The lysate is stable for 6 months at -20°C.

**Immunoprecipitation:** 4µg 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 phospho-tyrosine, add 0.2mM sodium orthovanadate to the lysis buffer.



### Immunoblot Analysis

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 **4µg of a-Phosphotyrosine** to 500µg-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100µl of washed Protein A agarose bead slurry (50µl packed beads).
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 50µl 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or boiled for 5 minutes. The beads are collected by a microcentrifuge pulse and SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.

### 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<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 (PBS-MLK) for 1 hour at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2µg/ml of a-Phosphotyrosine**, 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 IgG** linked to horseradish peroxidase, 1:2000 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 30 second exposure was used).