



cell signaling solutions

## Certificate of Analysis

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### Anti-Phosphotyrosine, recombinant 4G10

(mouse monoclonal IgG<sub>2bκ</sub>)

Catalog # 05-777

Lot # 27669

**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<sub>2bκ</sub> 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.<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. 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 orthovanadate 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  $1\mu\text{g}/\mu\text{l}$  total cell protein in a microcentrifuge tube with PBS.
2. Add **2-4 $\mu\text{g}$  of anti-Phosphotyrosine, recombinant 4G10**, to 500 $\mu\text{g}$ -1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 $\mu\text{l}$  (50 $\mu\text{l}$  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 70 $\mu\text{l}$  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; 1 $\mu\text{g}/\text{ml}$  aprotinin, leupeptin, pepstatin; 1mM  $\text{Na}_3\text{VO}_4$ ; 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 **0.5-2 $\mu\text{g}/\text{ml}$  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, 1:2000 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 10-30 second exposure was used).