



cell signaling solutions

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## Certificate of Analysis

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### Phosphotyrosine Control (EGF-stimulated A431 cell lysate) Catalog # 12-110 Lot # 23035

**Product Description:** Cellular protein preparation from A431 cells containing phosphorylated proteins, including the EGF receptor. A431 cells were cultured for 20 minutes in the presence of 50ng/ml EGF (Catalog # 01-107). Cells were lysed in modified RIPA buffer (50mM Tris-HCl, pH 7.4, 1% NP40, 0.25% sodium deoxycholate, 150mM NaCl, 1mM EDTA, 1mM PMSF, 1µg/ml aprotinin, 1µg/ml leupeptin, 1µg/ml pepstatin, 1mM Na<sub>3</sub>VO<sub>4</sub>, 1mM NaF).

**Formulation:** 1mg of cell lysate provided in 2 vials, each vial containing 500µg in 250µl of RIPA sample buffer. Concentration: 2µg/µl. Frozen solution.

**Storage and Stability:** Stable for 6 months at -20°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot to avoid repeated freezing and thawing.

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

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#### Quality Control Testing

Positive Control for Western Immunoblot Analysis: A previous lot of this preparation was tested by using anti-phosphotyrosine monoclonal antibody at 0.5-2µg/ml (Catalog # 05-321) to detect the phosphorylated EGF receptor (170kDa) and other tyrosine phosphorylated proteins in 10-50µg of the preparation.

**Use:** For immunoblot analysis, add an equal volume of Laemmli reducing sample buffer to 5-25µl of lysate and boil for 5 minutes to reduce the preparation.

#### Additional Research Applications

Immunoprecipitation Positive Control: This preparation may also be used for immunoprecipitation with anti-phosphotyrosine (4G10), agarose conjugate (Catalog # 16-101).

**Note:** This protein preparation may also be used as positive control for other Upstate Biotechnology Inc. antibodies.