

Certificate of Analysis

EGF Stimulated A431 Cell Lysate

Catalog # 12-302

Lot # DAM1547924

Product Description: Cellular protein preparation. Cells were cultured for 20 minutes in the presence of 50 ng/ml EGF (catalog #01-107), lysed in modified RIPA buffer (50mM Tris-HCl, pH 7.4, 1% NP40, 0.25% sodium deoxy-cholate, 150mM NaCl, 1mM EGTA, 1mM PMSF, 1µg/ml aprotinin, 1µg/ml leupeptin, 1µg/ml pepstatin, 1mM Na₃VO₄, 1mM NaF) and **diluted with non-reducing sample buffer** (31mM Tris-HCl, pH 6.8, 5% glycerol, 1% SDS, 0.002% bromophenol blue).

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. This preparation may be used as a positive control for some of Upstate Biotechnology's antibodies.

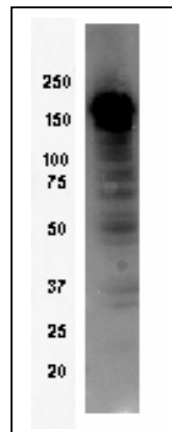
Quantity and Formulation: 100µg in 100µl of RIPA diluted with non-reducing sample buffer. Concentration: 1mg/ml. Frozen solution.

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**

Quality Control Testing

Immunoblot Analysis: 20µg of this lot of EGF Stimulated A431 Cell Lysate was tested by using 0.5-2µg/ml monoclonal anti-phosphotyrosine, clone 4G10® (Catalog # 05-321) to detect the tyrosine phosphorylated proteins.



Western Blot Analysis:

EGF-stimulated A431 cell lysate was resolved by electrophoresis, transferred to PVDF and probed with anti-phosphotyrosine (0.5 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system

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