

### Certificate of Analysis

**Product:** Human Derived EGF Stimulated A431 Whole Cell Lysate (Ready-to-Use)

**Code:** W09-000-362

**Lot #** 18741

**Size:** 500 µg (500 µl)

**Physical State:** Liquid (sterile filtered)

**Total Protein Concentration:** 1.0 mg/ml (by Lowry Assay)

**Buffer:** 1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8).

**Cell Line:** Human A431 (epidermoid carcinoma)      **Induction:** Epidermal Growth Factor (50 ng/ml)

**Background):** Ready-to-use whole cell lysates produced by Rockland Immunochemicals are derived from cell lines or tissues using highly refined extraction protocols to ensure exceptionally high quality, protein integrity and lot-to-lot reproducibility. All extracts are tested by SDS-PAGE using 4-20% gradient gels and immunoblot analysis using antibodies to key cell signaling components to confirm the presence of both high molecular weight and low molecular weight proteins.

**Application(s):** Ready-to-use lysates are especially prepared as positive controls for separation by SDS-PAGE and subsequent western blot analysis. Lysates are prepared in denaturing buffer WITHOUT dissociating agents (i.e. no 2-mercaptoethanol or dithiothreitol has been added).

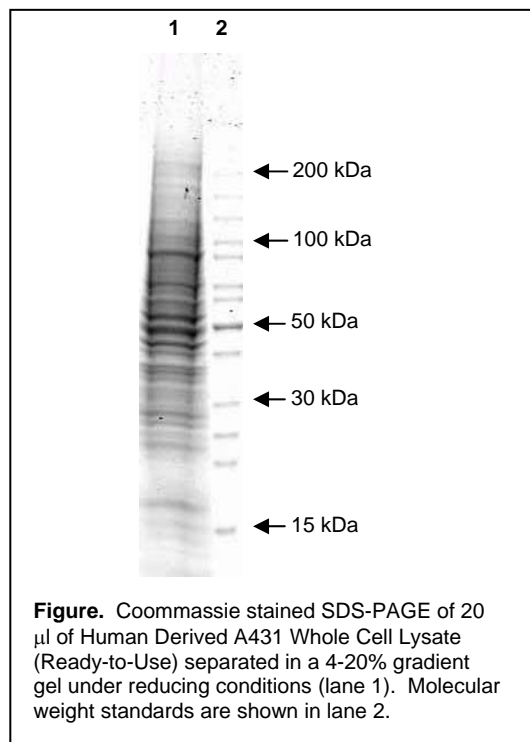
**Preparation Method:** The cells were grown in DMEM supplemented with 10% FBS (Fetal Bovine Serum). Cells were serum-starved for 1 h, followed by treatment with 50 ng/ml EGF for 15 min. Cells were washed in PBS and incubated on ice in modified RIPA buffer containing 150 mM sodium chloride, 50 mM Tris Cl, pH 7.4, 1 mM EDTA, 1.0% NP-40, 0.5% sodium deoxycholic acid and 0.1% SDS to lyse the cells. Protein integrity is ensured using a cocktail of protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases (0.1 mM AEBSF HCl, 0.08 µM Aprotinin, 5 µM Bestatin, 1.5 µM E-64, 2 µM Leupeptin Hemisulfate and 1 µM Pepstatin A). Phosphatase inhibitors sodium fluoride, sodium orthovanadate, sodium pyrophosphate and β-glycerophosphate were also added. Cell debris was removed by centrifugation. Protein concentration was determined by Lowry assay using a commercially available kit. The protein concentration was adjusted to 2 mg/ml and then an equal volume of 2X SDS-PAGE sample buffer was added.

**Recommended Dilution(s):** Heat lysate to 95° C for 5 minutes and rapidly cool. If dissociating conditions are desired add reducing agent prior to heating. The recommended loading volume per lane is 10-20 µl depending on the size format of your gel.

**Storage Conditions:** Store vial at -20° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles. Expiration date is three (3) months from date of opening if stored at -20° C or one (1) year from date of opening if stored at -70°C.

**Custom Service(s):** Please inquire for nuclear and/or whole cell extracts from other unstimulated or stimulated cell lines or normal tissues in both research and bulk quantities. Custom lysates from researcher-provided cell lines are also prepared using our highly refined extraction protocols. Please contact our Technical Service staff for additional details.

**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.



**Figure.** Coomassie stained SDS-PAGE of 20 µl of Human Derived A431 Whole Cell Lysate (Ready-to-Use) separated in a 4-20% gradient gel under reducing conditions (lane 1). Molecular weight standards are shown in lane 2.