

**Certificate of Analysis**

**Product:** Human Derived 293 Whole Cell Lysate (Ready-to-Use)

**Code:** W09-000-365

**Lot #** 16638

**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 (0.05 mM Tris HCl, 2% SDS, 10% Glycerol and 0.1% bromophenol blue, pH 6.8).

**Cell Line:** Human Epithelial (Kidney)

**Induction:** None (Control)

**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).

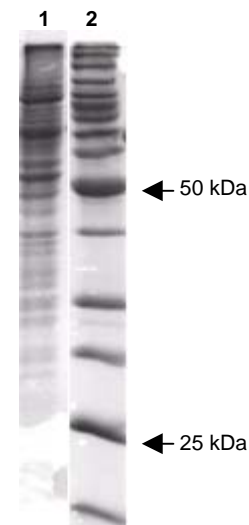
**Preparation Method:** The cells were grown in RPMI supplemented with 10% FBS (Fetal Bovine Serum). The lysate was prepared by first washing the cells in PBS supplemented with EDTA and a cocktail of protease inhibitors (see below). Washed cells are then incubated on ice in modified RIPA buffer containing 150 mM sodium chloride, 50 mM Tris Cl, pH 7.4, 1 mM EDTA, 1.0% NP-40, 0.25% sodium deoxycholic acid 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 (0.5 mM AEBSF HCl, 0.4 mM Aprotinin, 25 µM Bestatin, 7.5 µM E-64, 10 µM Leupeptin Hemisulfate and 5 µM Pepstatin A). Cell debris was removed by centrifugation. Protein concentration was determined by biuret assay using a commercially available kit. The protein concentration was adjusted to 5 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 -70° 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 (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 293 Whole Cell Lysate (Ready-to-Use) separated in a 4-20% gradient gel under non-reducing conditions (lane 1). Molecular weight standards are shown in lane 2.