

**Certificate of Analysis**

**Product:** Human Derived A431 Cell Nuclear Extract (Ready-to-Use)

**Code:** W09-001-A60

**Lot #** 19790

**Size:** 200 µg (200 µl)

**Physical State:** Liquid

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

**Buffer:** 1X SDS-PAGE Sample Buffer consisting of 50 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8.

**Cell Line:** Human A431 (epidermoid carcinoma)

**Induction:** None (Control)

**Background):** Ready-to-use nuclear extracts 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 nuclear extracts are especially prepared as positive controls for separation by SDS-PAGE and subsequent western blot analysis. Nuclear extracts are supplied in denaturing buffer without dissociating agents.

**Preparation Method:** The cells were grown in DMEM supplemented with 10% FBS (Fetal Bovine Serum). The lysate was prepared by first washing the cells in PBS. Washed cells were then incubated on ice in lysis buffer containing 10 mM HEPES, 60 mM KCl, 1.0 mM EDTA, 0.075% (v/v) NP40 and 1.0 mM DTT, pH 7.6. 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). Nuclei were then collected and washed in lysis buffer minus detergent. Nuclei were lysed by vortexing in extraction buffer containing 20 mM Tris-Cl, 1.5 mM MgCl<sub>2</sub>, 0.42 M NaCl, 0.2 mM EDTA, and 25% (v/v) glycerol, pH 8.0, supplemented with protease inhibitors (see above). The lysate was clarified by centrifugation. Protein concentration was determined by Lowry assay using a commercially available kit. The protein concentration was adjusted to 2.0 mg/ml and then an equal volume of 2X SDS-PAGE sample buffer was added.

**Recommended Dilution(s):** Heat nuclear extract 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 10-30 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 (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.

**Warning:** No test method can provide total assurance that the hepatitis B virus, hepatitis C virus, human immunodeficiency virus, or any other infectious agents are absent. Thus, all blood products, including purified proteins derived from human blood sources, should be handled at Biosafety Level 2 as recommended by the CDC/NIH manual entitled Biosafety in Microbiological and Biomedical Laboratories for potentially infectious human serum, blood specimens or proteins derived from same. Source material for the human blood product supplied to your facility has been tested for the detection of HIV antibody, Hepatitis B surface antigen, antibody to Hepatitis C, HIV 1 antigen(s), antibody to HTLV - I/II, and syphilis by FDA guidelines. All units were found to be non-reactive/negative for these tests. All human blood source material is collected in FDA licensed centers and is tested with FDA approved test kits.

**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.