

Human A431 Cell Nuclear Extract

Cat. No. A431-030HCL **Lot. No.** (See product label)

SPECIFICATION

Product Overview

Ready-to-use nuclear extracts 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.

Species

Human

Form

Liquid (sterile filtered)

Specificity

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 μ M Aprotinin, 5 μ M Bestatin, 1.5 μ M E-64, 2 μ M Leupeptin Hemisulfate and 1 μ M 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 MgCl₂, 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

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then an equal volume of 2× SDS-PAGE sample buffer was added.

Cell Line	Human A431 (epidermoid carcinoma)
Lysate Fractionation	Nuclear Extract
Lysate Stimulation	Not Stimulated
Culture Type	Tissue Culture
Induction	None (Control)
Concentration	1.0 mg/mL by modified Lowry assay
Buffer	1× SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Preservative	None
Shipping	Dry Ice
Storage	Store vial at -70 centigrade or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Expiration	Expiration date is three months from date of receipt.

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