Human A431 Cell Nuclear Extract

Cat. No. A431-2H **Lot. No.** (See product label)

SPECIFICATION

Species	Human
Concentration	2 mg/ml
Tissue Type	A431 (epidermoid carcinoma)
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 M Aprotinin, 5 M Bestatin, 1.5 M E-64, 2 M Leupeptin Hemisulfate and 1 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 then an equal volume of 2X SDS-PAGE sample buffer was added.
Recommended Usage	For research use only, not for diagnostic or therapeutic use.
Storage Buffer	1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and

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	0.005% bromophenol blue, pH 6.8)
Applications	Protein Lysate for WB

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