Human Doxorubicin Stimulated HeLa Cell Nuclear Extract

Cat. No. HeLa-035HCL **Lot. No.** (See product label)

SPECIFICATION

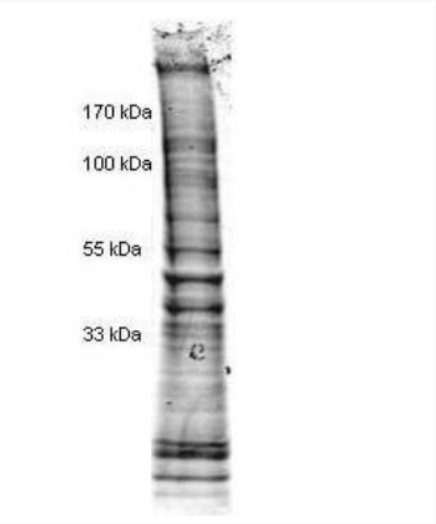
Product Overview	Ready-to-use nuclear extracts are derived from cell lines or tissues using highly refined extraction protocols to ensure exceptionally high quality, protein integrity and lot-to-lot reproducibility. All extracts are tested by SDS-PAGE using 4-20% gradient gels and immunoblot analysis 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). Cells were treated with 0.5 µg/mL Doxorubicin for 2 h. 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 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

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	concentration was adjusted to 2.0 mg/mL and then an equal volume of 2× SDS-PAGE sample buffer was added.
Applications	SDS-PAGE
Cell Line	HeLa - Human epidermoid carcinoma
Lysate Fractionation	Nuclear Extract
Lysate Stimulation	Doxorubicin
Culture Type	Tissue Culture
Induction	Doxorubicin (0.5 µg/mL)
Concentration	1.0 mg/mL by BCA 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
Stabilizer	10% (v/v) Glycerol
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.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

SDS-PAGE



Coomassie stained SDS-PAGE of 25 µg of Doxorubicin stimulated Human Derived Hela Cell Nuclear Extract (Ready-to-Use) separated in a 4-20% gradient gel under non-reducing conditions. Molecular weight standards are shown on the left.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA