HeLa Cell Nuclear Extract

Cat. No. Hela-01HL **Lot. No.** (See product label)

SPECIFICATION

Product Overview	HeLa Cell Nuclear Extract
Species	Human
Source	HeLa Nuclear Extract (epidermoid carcinoma)
Lysate preparation	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 MgCl2, 0.42 M NaCl, 0.2 mM EDTA, and 25% (v/v) glycerol, pH 8.0, supplemented with protease inhibitors. 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 2× SDS-PAGE sample buffer was added.
Recommended Usage	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

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	(i.e. no 2- mercaptoethanol or dithiothreitol has been added).
Storage	Store at 2-8 centigrade for continuous use. For extended storage, freeze working aliquots at -70 centigrade. Repeated freezing and thawing is not recommended. Under proper storage conditions the shelf life is half a year from the date of receipt.
Storage Buffer	1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Concentration	2 mg/mL
SDS-PAGE	

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