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Datasheet for W09-001-A83

HeLa Cell Nuclear Extract Etoposide Stimulated

Overview

Description:	HeLa Cell Nuclear Extract - Etoposide Stimulated - W09-001-A83
Item No.:	W09-001-A83
Size:	200 µg
Applications:	SDS-PAGE
Origin:	Human

Product Details

Background:	Ready-to-use HeLa Cell Nuclear Extract Etoposide Stimulated 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.
Synonyms:	HeLa Cell Nuclear Extract Etoposide Stimulated, HeLa Etoposide Stimulated Nuclear Lysate, HeLa Nuclear Lysate Etoposide Stimulated, HeLa Etoposide Stimulated Nuclear Extract
Species of Origin:	Human

Target Details

Purity/Specificity:	The cells were grown in Eagles medium supplemented with 10% FBS (Fetal Bovine Serum). Cells were treated with 10 µg/ml Etoposide for 13 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 concentration was adjusted to 2.0 mg/ml and then an equal volume of 2X SDS-PAGE sample buffer was added.

Application Details

Tested Applications:	SDS-PAGE
Application Note:	W09-001-A83 has been tested by SDS-PAGE. 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. 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 ul depending on the size format of your gel.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
WB:	User Optimized

Cell Line Data

Cell Line:	HeLa - Human epidermoid carcinoma
Lysate Fractionation:	Nuclear Extract
Lysate Stimulation:	Etoposide
Culture Type:	Tissue Culture
Induction:	Etoposide (10 µg/ml)

Formulation

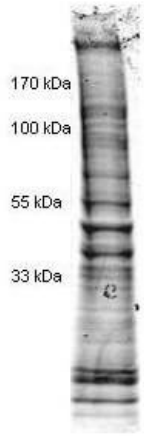
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by modified Lowry assay
Buffer:	1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Preservative:	None
Stabilizer:	10% (v/v) Glycerol

Shipping & Handling

Shipping Condition:	Dry Ice

Storage Condition:	Store HeLa Cell Nuclear Extract Etoposide Stimulated at -70° C or COLDER. For extended storage, aliquot Nuclear Extract to minimize freeze/thaw cycles.
Expiration:	Expiration date is three (3) months from date of receipt.

Images



SDS-PAGE
Coomassie stained SDS-PAGE of 25 ?g of Etoposide 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.

Disclaimer

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. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.