

# Human Etoposide Stimulated HeLa Cell Nuclear Extract

**Cat. No.** HeLa-036HCL    **Lot. No.** (See product label)

## SPECIFICATION

**Product Overview**

Ready-to-use HeLa Cell Nuclear Extract Etoposide Stimulated 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 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 MgCl<sub>2</sub>, 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

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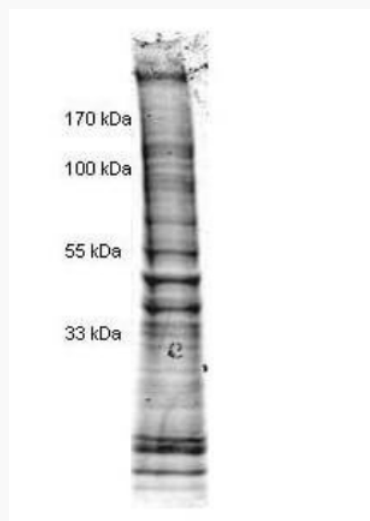
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|                             |                                                                                                                                                                 |
|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                             | kit. The protein concentration was adjusted to 2.0 mg/mL and then an equal volume of 2× SDS-PAGE sample buffer was added.                                       |
| <b>Applications</b>         | SDS-PAGE                                                                                                                                                        |
| <b>Cell Line</b>            | HeLa - Human epidermoid carcinoma                                                                                                                               |
| <b>Lysate Fractionation</b> | Nuclear Extract                                                                                                                                                 |
| <b>Lysate Stimulation</b>   | Etoposide                                                                                                                                                       |
| <b>Culture Type</b>         | Tissue Culture                                                                                                                                                  |
| <b>Induction</b>            | Etoposide (10 µg/mL)                                                                                                                                            |
| <b>Concentration</b>        | 1.0 mg/mL by modified Lowry assay                                                                                                                               |
| <b>Buffer</b>               | 1× SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)                                                          |
| <b>Preservative</b>         | None                                                                                                                                                            |
| <b>Stabilizer</b>           | 10% (v/v) Glycerol                                                                                                                                              |
| <b>Shipping</b>             | Dry Ice                                                                                                                                                         |
| <b>Storage</b>              | Store HeLa Cell Nuclear Extract Etoposide Stimulated at -70 centigrade or COLDER. For extended storage, aliquot Nuclear Extract to minimize freeze/thaw cycles. |
| <b>Expiration</b>           | Expiration date is three months from date of receipt.                                                                                                           |

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**SDS-PAGE**

Coomassie stained SDS-PAGE of 25 µg 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.

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