

Human HeLa Whole Cell Lysate

Cat. No. HeLa-003HCL **Lot. No.** (See product label)

SPECIFICATION

Product Overview

Ready-to-use HeLa whole cell lysates are derived from cell lines 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

Specificity

The HeLa cells were grown in medium supplemented with 10% fetal bovine serum. Cells were washed with PBS and then incubated on ice in modified RIPA buffer to lyse the cells. Protein integrity was 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 μ M Aprotinin, 5 μ M Bestatin, 1.5 μ M E-64, 2 μ M Leupeptin Hemisulfate, 1 μ M Pepstatin A). Phosphatase inhibitors 1 mM NaF and 1 mM Na₃VO₄ were also added. Cell debris was removed by centrifugation. Protein concentration was determined by a modified Lowry assay using a commercially available kit. Protein concentration was adjusted to 2 mg/mL and then an equal volume of 2× SDS-PAGE sample buffer was added.

Applications

WB, IF, SDS-PAGE, Cellular Assay, Other

Cell Line

HeLa - Human epidermoid carcinoma

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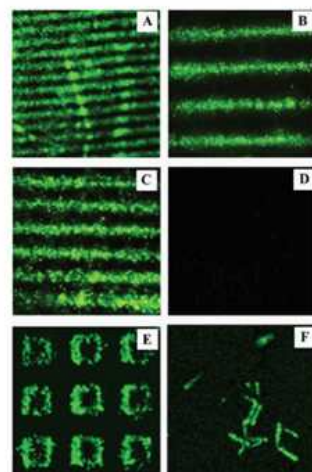
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Lysate Fractionation	Whole Cell Lysate
Lysate Stimulation	Not Stimulated
Culture Type	Tissue Culture
Induction	None (Control)
Concentration	1.0 mg/mL by BCA assay
Buffer	1× SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol 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.
References	1. Dorfman A et al. Novel telomeric repeat elongation assay performed on zinc oxide nanorod array supports. J Nanosci Nanotechnol. (2008)

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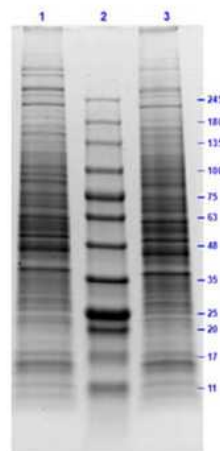
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Immunofluorescence Microscopy

Fluorescence images acquired from TRE assay performed on ZnO NR arrays. The fluorescence images are (A) $1200 \times 1200 \mu\text{m}$, (B) $400 \times 400 \mu\text{m}$, (C) $120 \times 120 \mu\text{m}$, (D) $120 \times 120 \mu\text{m}$, (E) $60 \times 60 \mu\text{m}$, and (F) $25 \times 25 \mu\text{m}$ in size. The underlying ZnO NR platforms used in assays A through D have striped patterns. (A–C) Confocal fluorescence data obtained from positive samples. The green fluorescence emission is clearly seen in these samples which signifies the detection of active telomerase and the successful incorporation of dNTPs in the extension of TS. (D) Typical fluorescence image collected from negative samples. No fluorescence emission is detected from these samples due to the lack of telomerase in the assay. The platforms used in assays E and F are open square and individual ZnO NRs, respectively. (E and F) Fluorescence images obtained from positive samples on (E) open square ZnO NR and (F) individual ZnO NR arrays. Negative assays were carried out by omitting either HeLa cell lysates or dNTPs.

SDS-PAGE



SDS PAGE Results of HeLa Whole Cell Lysates.

Lane 1: HeLa Whole Cell Lysate Reduced [10 µg].

Lane 2: Opal Prestained Molecular Weight Marker.

Lane 3: HeLa Whole Cell Lysate Non-Reduced [10 µg].

4-20% Gel, Coomassie Stained.

Results show wide range of molecular weight bands with no signs of degradation.

SDS-PAGE

Coomassie stained SDS-PAGE of 10 µg of Human Derived HeLa Whole Cell Lysate separated using a 4-20% gradient gel under reducing conditions (lane 2). Molecular weight standards are shown in lane 1.

Western Blot

Western Blot showing detection of alpha tubulin in lane 1. HeLa Whole Cell Lysate (10 µg) was run on a 4-20% gel, then transferred to 0.45 µm nitrocellulose. After blocking with 1% BSA-TTBS for 30 min at 20 centigrade, primary antibody was used

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at 1:2500 overnight at 4 centigrade. Anti Rabbit IgG (H&L) (GOAT) antibody IRDye800CW secondary antibody was used at 1:20,000 with Blocking Buffer for Fluorescent Western Blotting and imaged on the LiCor Odyssey imaging system. Arrow indicates correct 50 kDa molecular weight position expected for alpha tubulin.

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