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Datasheet for W09-000-364

HeLa Whole Cell Lysate

Overview

Description:	HeLa Whole Cell Lysate - W09-000-364
Item No.:	W09-000-364
Size:	500 µg
Applications:	SDS-PAGE, WB, Cellular Assay, IF, Other
Origin:	Human

Product Details

Background:	Ready-to-use HeLa whole cell lysates produced by Rockland Immunochemicals 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.
Synonyms:	Hela Cells, HeLa Lysate, Human Derived Whole Cell Lysate, Human Derived HeLa Whole Cell Lysate
Species of Origin:	Human

Target Details

Purity/Specificity:	The Hela cells were grown in Dulbecco's 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 µM Aprotinin, 5 µM Bestatin, 1.5 µM E-64, 2 µM Leupeptin Hemisulfate, 1 µM 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 2X SDS-PAGE sample buffer was added.

Application Details

Tested Applications:	SDS-PAGE, WB

Suggested Applications:	Cellular Assay, IF, Other (Based on references)
Application Note:	W09-000-364 has been tested by SDS-PAGE and western blot. Ready-to-use HeLa whole cell 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 (i.e. no 2-mercaptoethanol or dithiothreitol has been added). Heat lysate 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 10-20 µl 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:	Whole Cell Lysate
Lysate Stimulation:	Not Stimulated
Culture Type:	Tissue Culture
Induction:	None (Control)

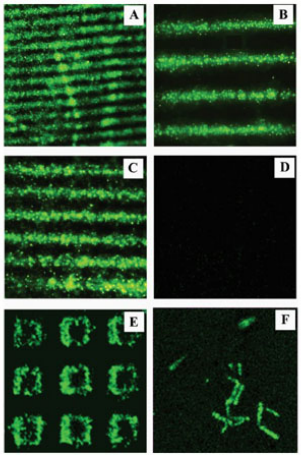
Formulation

Physical State:	Liquid
Concentration:	1.0 mg/mL by BCA 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

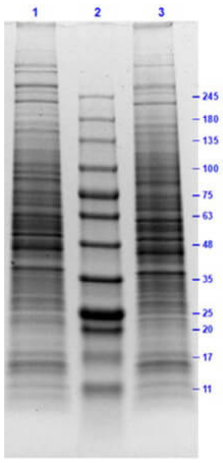
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Expiration:	Expiration date is three (3) months from date of receipt.

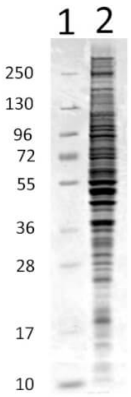
Images



Immunofluorescence Microscopy
Fluorescence images acquired from TRE assay performed on ZnO NR arrays. The fluorescence images are (A) 1200 × 1200 m, (B) 400 × 400 m, (C) 120 × 120 m, (D) 120 × 120 m, (E) 60 × 60 m, and (F) 25×25 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 (p/n W09-000-364) or dNTPs. Fig 3. PMID: 18468092.



SDS-PAGE
SDS PAGE Results of HeLa Whole Cell Lysates. Lane 1: HeLa Whole Cell Lysate Reduced [10µg]. Lane 2: Opal Prestained Molecular Weight Marker (p/n MB-210-0500). Lane 3: HeLa Whole Cell Lysate Non-Reduced [10µg]. 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 (p/n MB-013, diluted to 1X) for 30 min at 20°C, primary antibody was used at 1:2500 overnight at 4°C. Anti-Rabbit IgG (H&L) (GOAT) antibody IRDye800CW® (p/n 611-131-002) secondary antibody was used at 1:20,000 with Blocking Buffer for Fluorescent Western Blotting (p/n MB-070) and imaged on the LiCor Odyssey imaging system. Arrow indicates correct 50 kDa molecular weight position expected for alpha tubulin.

References

- Dorfman A et al. Novel telomeric repeat elongation assay performed on zinc oxide nanorod array supports. *J Nanosci Nanotechnol.* (2008)

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