HeLa Whole Cell Lysate

Cat#: orb348668 (User Manual)

Product Description: HeLa Whole Cell Lysate
Concentration: 1.0 mg/mL by BCA assay
Physical State: Liquid
Label Unconjugated
Buffer 1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Stabilizer 10% (v/v) Glycerol
Preservative None
Storage Condition Store vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Synonyms Hela Cells, HeLa Lysate, Human Derived Whole Cell Lysate, Human Derived HeLa Whole Cell Lysate

Application Note 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.

Background Ready-to-use HeLa whole cell lysates produced by Rockland Immunochemical 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.

Purity and 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, containing 150 mM sodium chloride, 50 mM Tris HCl, pH 7.4, 1 mM EDTA, 1.0% NP-40, 0.5% sodium deoxycholic acid, 0.1% SDS and 0.01% (w/v) sodium azide 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.

Assay Dilutions User Optimized
Western Blot User Optimized
Other Assays User Optimized

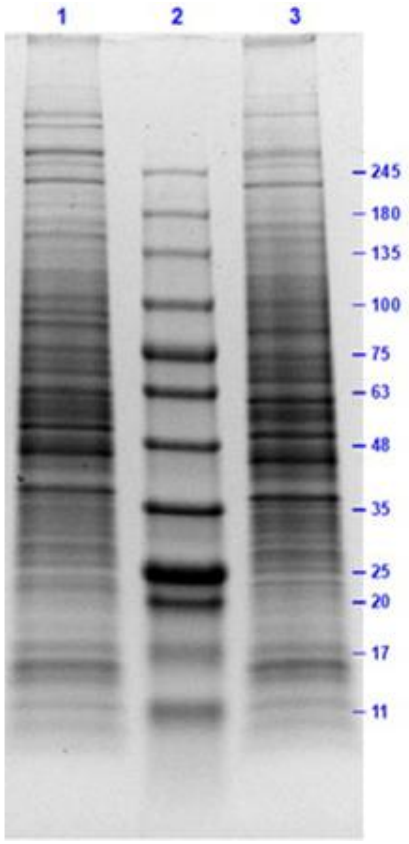
Biorbyt Ltd.
5 Orwell Furlong, Cowley Road,Cambridge, Cambridgeshire
CB4 0WY, United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859 353 | Fax: +44(0)1223 280 240

Biorbyt LLC
Suite 103, 369 Pine Street, San Francisco
California 94104, United States
Email: info@biorbyt.com | Phone: +1 (415)-906-5211 | Fax: +1 (415) 651 8558

Expiration Expiration date is three (3) months from date of opening.

Related Products
200-301-268 Anti-AKT pS473 (MOUSE) Monoclonal Antibody - 200-301-268 610-4302 Anti-MOUSE IgG (H&L) (RABBIT) Antibody Peroxidase Conjugated - 610-4302 611-1302 Anti-RABBIT IgG (H&L) (GOAT) Antibody Peroxidase Conjugated - 611-1302 B304 NORMAL GOAT SERUM (NGS) - B304

Images
1 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.



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% BSATTBS (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.

Coomassie stained SDS-PAGE of 20 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.

Biorbyt Ltd.
5 Orwell Furlong, Cowley Road,Cambridge, Cambridgeshire
CB4 0WY, United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859 353 | Fax: +44(0)1223 280 240

Biorbyt LLC
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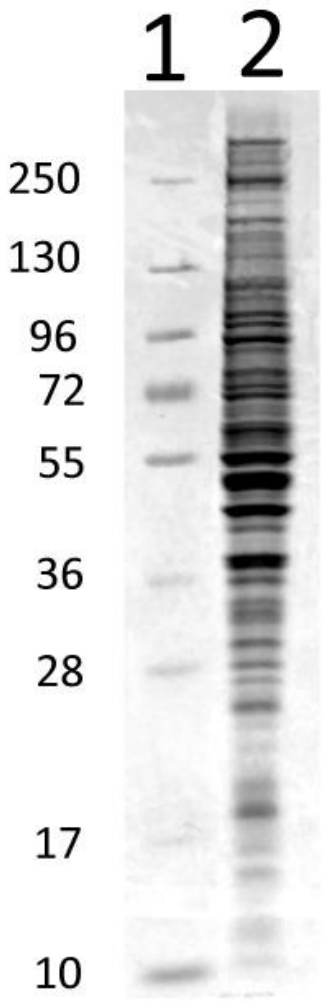
California 94104, United States
Email: info@biorbyt.com | Phone: +1 (415)-906-5211 | Fax: +1 (415) 651 8558



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