HeLa Whole Cell Lysate - W09-000-364

Code: W09-000-364 **Size:** 500 µg

Product Description: HeLa Whole Cell Lysate - W09-000-364

Concentration: 1.0 mg/mL by modified Lowry assay

PhysicalState: Liquid

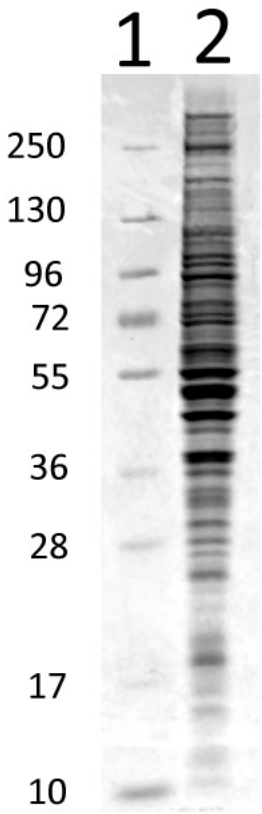
Label	Unconjugated
Buffer	1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Stabilizer	10% (v/v) Glycerol
Preservative	None
Storage Condition	Store vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Synonyms	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 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.
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
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	Coommassie 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.



2 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.

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.