

Datasheet for W09-001-GY4**U-251 Whole Cell Lysate****Overview**

Description:	U-251 Whole Cell Lysate - W09-001-GY4
Item No.:	W09-001-GY4
Size:	500 µg
Applications:	SDS-PAGE, WB
Origin:	Human

Product Details

Background:	Ready-to-use whole cell lysates produced by Rockland Immunochemicals 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.
Synonyms:	U251 Whole Cell Lysate, U-251 WCL, U-251 Lysate, U-251 MG Lysate, U-251-MG Lysate, U-251_MG Lysate, U251-MG Lysate, U251MG, U251N
Species of Origin:	Human

Target Details

Purity/Specificity:	U-251 cells were grown in Eagle's Minimum Essential 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 BCA assay using a commercially available kit. Protein concentration was adjusted to 1 mg/mL using 2X SDS-PAGE Sample buffer.
----------------------------	--

Application Details

Tested Applications:	SDS-PAGE, WB
-----------------------------	--------------

Application Note:	W09-001-GY4 has been tested by SDS-PAGE and western blot. Ready-to-use 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:	Human - Astrocytoma
Lysate Fractionation:	Whole Cell Lysate
Lysate Stimulation:	Not Stimulated
Culture Type:	Tissue Culture
Induction:	None (Control)

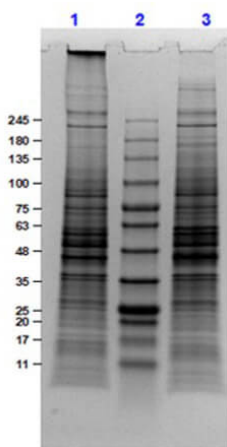
Formulation

Physical State:	Liquid (sterile filtered)
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

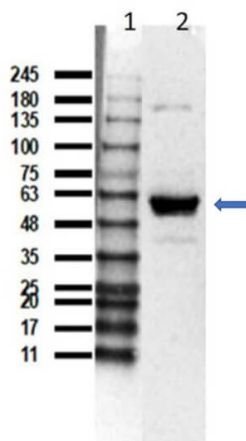
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Expiration:	Expiration date is three (3) months from date of receipt.

Images



SDS-PAGE

SDS-PAGE Results of U-251 Whole Cell Lysate. Lane 1: U-251 Whole Cell Lysate (10µg) Reduced. Lane 2: Opal Prestained Molecular Weight Marker (p/n MB-210-0500). Lane 3: U-251 Whole Cell Lysate (10µg) Non-Reduced. Results show wide range of molecular weight bands with no sign of degradation.



Western Blot

Western Blot results of Rabbit Anti-Beclin 1 Antibody using U-251 lysate. Lane 1: Opal Prestain Molecular Weight Marker (p/n MB-210-0500). Lane 2: U251-MG whole cell lysate (35ug) [+]. Primary Antibody: Anti-Beclin1 at 1:1000 overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG HRP (p/n 611-103-122) at 1:40,000 for 60min at RT. Block: 5% BLOTTO/TBST (p/n B501-0500). Expected MW: ~51kDa. Observed MW: ~53kDa.

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.