

**Datasheet for W09-001-GX2****U-87 MG Whole Cell Lysate****Overview**

<b>Description:</b>	U-87 MG Whole Cell Lysate - W09-001-GX2
<b>Item No.:</b>	W09-001-GX2
<b>Size:</b>	500 µg
<b>Applications:</b>	SDS-PAGE, WB
<b>Origin:</b>	Human

**Product Details**

<b>Background:</b>	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.
<b>Synonyms:</b>	U-87 WCL, U87 Whole Cell Lysate, U-87 Lysate
<b>Species of Origin:</b>	Human

**Target Details**

<b>Purity/Specificity:</b>	U-87 MG 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.
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**Application Details**

<b>Tested Applications:</b>	SDS-PAGE, WB
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<b>Application Note:</b>	W09-001-GX2 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.
<b>Assay Dilutions:</b>	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
<b>WB:</b>	User Optimized

## Cell Line Data

<b>Cell Line:</b>	Human - Epithelial - likely glioblastoma (Brain)
<b>Lysate Fractionation:</b>	Whole Cell Lysate
<b>Lysate Stimulation:</b>	Not Stimulated
<b>Culture Type:</b>	Tissue Culture
<b>Induction:</b>	None (Control)

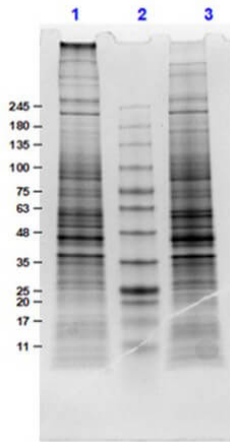
## Formulation

<b>Physical State:</b>	Liquid (sterile filtered)
<b>Concentration:</b>	1.0 mg/ml by BCA assay
<b>Buffer:</b>	1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
<b>Preservative:</b>	None
<b>Stabilizer:</b>	10% (v/v) Glycerol

## Shipping & Handling

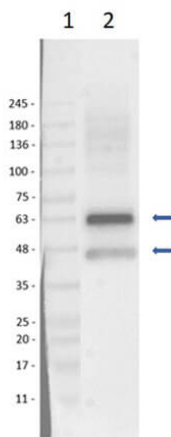
<b>Shipping Condition:</b>	Dry Ice
<b>Storage Condition:</b>	Store vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
<b>Expiration:</b>	Expiration date is three (3) months from date of receipt.

## Images



#### SDS-PAGE

SDS-PAGE Results of U-87 MG Whole Cell Lysate. Lane 1: U-87 MG Whole Cell Lysate (10µg) Reduced. Lane 2: Opal Prestained Molecular Weight (p/n MB-210-0500). Lane 3: U-87 MG Whole Cell Lysate (10µg) Non-Reduced. Results show wide range of molecular weight bands with no signs of degradation.



#### Western Blot

Western Blot Results of Rabbit Anti-SQSTM1/p62 Antibody using U-87 MG Lysate. Western Blot of Rabbit Anti-SQSTM1/p62 Antibody. Lane 1: Opal Prestain Molecular Weight Marker (p/n MB-210-0500). Lane 2: U-87 MG whole cell lysate. Load: 35µg lysate per lane. Primary Antibody: Anti-SQSTM1 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 60 mins at RT. Block: 5% BLOTTO/TBST (p/n B501-0500). Predicted MW: ~48 kDa. Observed MW: ~45, 60kDa. Protein undergoes acetylation, Isopeptide bond, Phosphoprotein, and Ubl conjugation.

## Disclaimer

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