

## U-87 MG Whole Cell Lysate - W09-001-GX2

Code: W09-001-GX2 Size: 500 µg

Product Description: U-87 MG Whole Cell Lysate - W09-001-GX2

Concentration: 1.0 mg/ml by BCA assay

PhysicalState: Liquid (sterile filtered)

Label Unconjugated

**Buffer** 1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH

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** U-87 WCL, U87 Whole Cell Lysate, U-87 Lysate

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-**Application Note** 

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 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.

**Purity And Specificity** 

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, 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 and 0.01% (WV) sodium azide to tyse the cells. Protein integrity was ensured using a cocktall of protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases (0.1 mM AEBSF HCI, 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.

Western Blot User Optimized

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

**Related Products** 

W09-000-361 A431 Whole Cell Lysate - W09-000-361

MB-210-0500 Opal Prestained Protein Standard 10-245kDaMB-210-0500

W09-001-GL5 A-172 Whole Cell LysateW09-001-GL5(1)

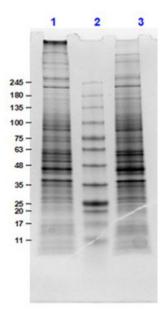
600-401-HB8 SQSTM1/p62 Antibody600-401-HB8

**Images** 

SDS-PAGE Results of U-87 MG Whole Cell Lysate. Lane 1: U-87

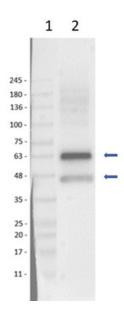
MG Whole Cell Lysate (10µg) Reduced. Lane 2: Opal Prestained Molecular Weight (p/n MB-210-0500). Lane 3: U-87 MG Whole Cell Lysate (10µg) Non-Reduced. Results show wide range of

molecular weight bands with no signs of degradation.



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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 lgG 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

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.

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