

Datasheet for W10-001-377**NIH/3T3 Whole Cell Lysate PDGF Stimulated****Overview**

Description:	NIH/3T3 Whole Cell Lysate - PDGF Stimulated - W10-001-377
Item No.:	W10-001-377
Size:	200 µg
Applications:	SDS-PAGE, WB
Origin:	Mouse

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:	NIH/3T3 Whole Cell Lysate PDGF Stimulated, NIH/3T3 WCL PDGF Stimulated, NIH/3T3 PDGF Stimulated Whole Cell Lysate, NIH/3T3 PDGF Stimulated WCL, NIH/3T3 Lysate PDGF Stimulated, NIH3T3 Lysate PDGF Stimulated
Species of Origin:	Mouse

Target Details

Purity/Specificity:	The 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 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.
----------------------------	---

Application Details

Tested Applications:	SDS-PAGE, WB
Application Note:	W10-001-377 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:	NIH Swiss Webster Mouse embryo
Lysate Fractionation:	Whole Cell Lysate
Lysate Stimulation:	PDGF
Culture Type:	Tissue Culture
Induction:	Platelet Derived Growth Factor (100 ng/ml)

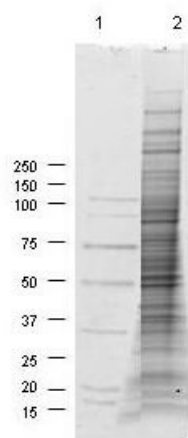
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by modified Lowry 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

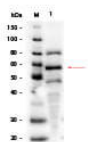
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Expiration:	Expiration date is three (3) months from date of receipt.

Images



SDS-PAGE

Coomassie stained SDS-PAGE of 30 µg of Mouse Derived NIH/3T3 Whole Cell Lysate (Ready-to-Use) resolved on a 4-20% gradient gel under non-reducing conditions (lane 2). Molecular weight standards are shown in lane 1.



Western Blot

Western Blot of NIH/3T3 Whole Cell Lysate PDGF Stimulated.

Lane 1: NIH/3T3 Whole Cell Lysate PDGF Stimulated.

Load: 2 µg per lane. Primary antibody: AKT1 pS473 Antibody at 1:1,000 overnight at 4°C. Secondary antibody: HRP mouse secondary antibody at 1:40,000 for 30 min at RT. Block: MB-070 for 30 min at RT. Predicted/Observed size: 55 kDa, 55 kDa for AKT1 pS473.

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.