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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)

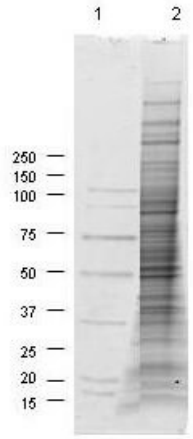
Formulation

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

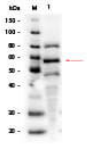
Shipping & Handling

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

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