Datasheet for ABIN964052

NIH/3T3 Whole Cell Lysate (PDGF Stimulated)

2 Images

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Overview

Quantity:	200 µg
Protein Species:	Mouse
Species of Lysate:	Mouse
Application:	Western Blotting (WB)

Product Details

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, 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 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.
Characteristics:	Cell Line: NIH Swiss Webster Mouse embryo Induction: Platelet Derived Growth Factor (100 ng/ml)
Sterility:	Sterile filtered
Lysate Fraction:	Whole Cell Lysate
Lysate Treatment:	PDGF Stimulated

Product Details

Lysate Type:	Cell Lysate
Lysed Cells:	3T3 Cells

Target Details

Background:	Ready-to-use whole cell lysates produced 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.

Application Details

Application Notes:	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.
Comment:	Lysate Fractionation: Whole Cell Lysate Lysate Stimulation: PDGF Lysate Tissue Culture: Tissue Culture
Restrictions:	For Research Use only

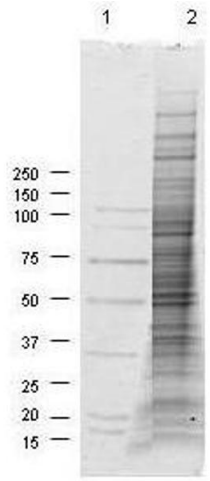
Handling

Format:	Liquid
Concentration:	1.0 mg/mL
Buffer:	1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Handling Advice:	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

Handling

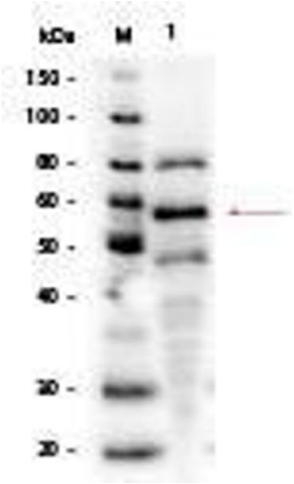
	format of your gel.
Storage:	-80 °C
Expiry Date:	3 months

Images



Western Blotting

Image 1.



Western Blotting

Image 2. 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: ABIN925618 for 30 min at RT. Predicted/Observed size: 55 kDa, 55 kDa for AKT1 pS473.