

Datasheet for ABIN964052

**NIH/3T3 Whole Cell Lysate (PDGF Stimulated)****2** Images[Go to Product page](#)

## 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 Na<sub>3</sub>VO<sub>4</sub> 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

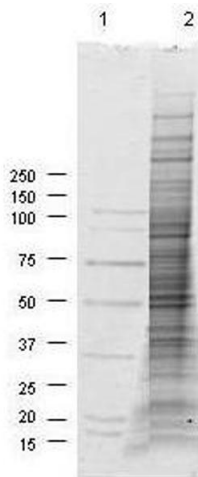
## Handling

format of your gel.

Storage: -80 °C

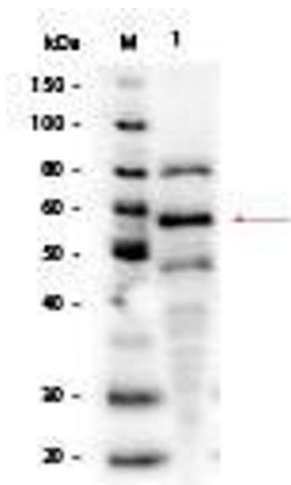
Expiry Date: 3 months

## 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  $\mu$ g 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.