abcam

Product datasheet

HeLa Signaling Cascade Whole Cell Lysate Set: PMA-Treated and Vehicle-Treated Control ab170195

2 Images

Overview

Product name HeLa Signaling Cascade Whole Cell Lysate Set: PMA-Treated and Vehicle-Treated Control

Product overview HeLa cells are an adherent human epithelial cell line derived from a cervical carcinoma. HeLa cells were lysed with 1% SDS in the prescence of protease and phosphatase inhibitors. Protein concentration of the extract was determined by BCA assay. The extract is suitable for use in SDS-PAGE and Western blotting.

PMA-treated HeLa lysate is designed for use as a western blot positive control when studying signaling cascades downstream of PKC. Cells were treated with 200 nM PMA for 1 hour after overnight serum starvation. Untreated cells were grown under the same conditions and treated instead with DMSO (PMA diluent as used to generate the treated lysate).

Concentration: HeLa PMA-treated lysate, 200 µg at 2.0 mg/mL
HeLa DMSO-treated lysate, 200 µg at 2.0 mg/mL

Notes PMA (phorbol 12-myristate 13-acetate) also known as TPA (12-O-Tetradecanoylphorbol 13-acetate), is a potent activator of protein kinase C (PKC), a calcium-activated, phospholipid- and diacylglycerol (DAG)-dependent serine/threonine-protein kinase. PKC is involved in positive and negative regulation of cell proliferation, apoptosis, differentiation, migration and adhesion, tumorigenesis, cardiac hypertrophy, angiogenesis, platelet function and inflammation, by directly phosphorylating targets such as RAF1, BCL2, CSPG4, TNNT2/CTNT, or activating signaling cascade involving MAPK1/3 (ERK1/2) and RAP1GAP.

Tested applications **Suitable for:** SDS-PAGE, WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	2 units
Control for PMA-Treated HeLa Lysate	1 x 200µg

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PMA-Treated HeLa Lysate	1 x 200µg

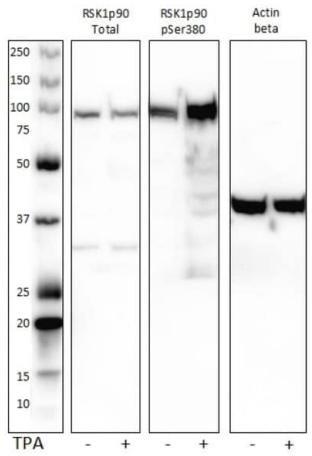
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of **ab170195** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

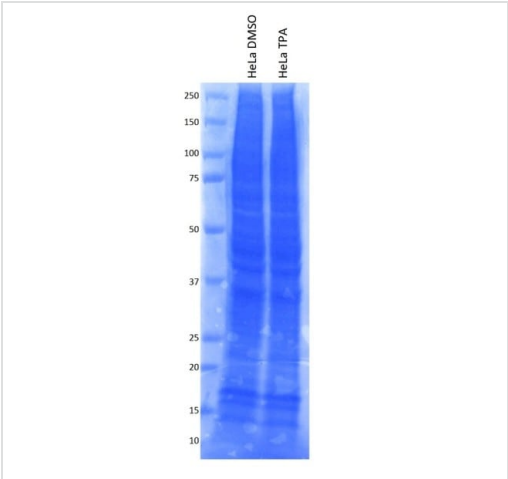
Application	Abreviews	Notes
SDS-PAGE		Use at an assay dependent concentration. Load 20 µg – 40 µg per lane Heat sample at 70°C before loading
WB		Use at an assay dependent concentration. Load 20 µg – 40 µg per lane Heat sample at 70°C before loading

Images



Western Blot Validation

HeLa cell cultures were serum-starved overnight and treated with 200nM of PMA for 1 hour at 37°C. 40µg of cell lysate was run on a 4-20% gradient gel then transferred to a PVDF membrane. All blocking and antibody incubation steps were done in 5% milk, 20 mM Tris-HCl, 0.1% TWEEN-20. Primary antibodies: RSK1p90 total: 1:1000 ab32114 RSK1p90 pSer380: 1:500 ab32203 Actin beta: 1µg/mL ab8226 Secondary antibodies: Goat polyclonal to Mouse IgG – H&L – Pre-Adsorbed (HRP) at 1/10000. Goat polyclonal to Rabbit IgG – H&L – Pre-Adsorbed (HRP) at 1/10000.



Optiblot Blue stain

Optiblot Blue stain HeLa cell cultures were serum-starved overnight and treated with 200 nM of PMA for 1 hour at 37°C. 40 µg of cell lysate was run on a 4-20% gradient gel then stained with Optiblot Blue (ab119211) for 2 hours at room temperature. Gel was destained using nanopure water for 24 hours.

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