abcam

Product datasheet

Cell Fractionation Kit - Standard ab109719

★★★★★ <u>4 Abreviews</u> <u>106 References</u> 3 Images

Overview

Product name Cell Fractionation Kit - Standard

Assay type Direct

Product overview Abcam's Cell Fractionation Kit (Standard) allows for the rapid and simple preparation of

mitochondrial, cytoplasmic and nuclear containing fractions from cultured cells from mammals and

other species.

The Cell Fractionation Kit is designed to produce highly enriched fractions that allow for the monitoring of proteins of interest through various cellular compartments. It is not

designed to produce purified cellular fractions.

This kit does not require mechanical disruption of the sample. This is important as mechanical disruption can disrupt mitochondrial membranes, causing the biologically-irrelevant release of proteins. This kit is particularly useful in studying apoptosis and the movement of pro-apoptotic proteins such as cytochrome c.

Sufficient materials are provided for fractionation of 1 \times 10⁸ cells or for preparation of 40 samples, each corresponding to one 100 mm plate at 2.5 \times 10⁶ cells/plate.

A companion product is also available which allows for the fractionation of cells grown in 96-well plates: Cell Fractionation Kit HT (<u>ab109718</u>).

Notes

Properties

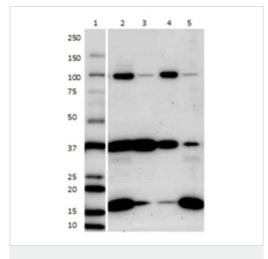
Storage instructions Please refer to protocols.

Components	1 kit
5X SDS Sample Buffer	1 x 10ml
Buffer A 2x concentrate	1 x 175ml
Detergent I	1 x 25µl

1

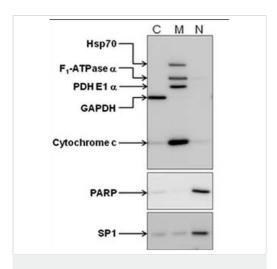
Components	1 kit
Detergent II	1 x 1ml

Images



Analysis of fractions by Western blot with a plasma membrane antibody cocktail.

Samples were loaded as follows from left to right: (1) Marker, (2) whole cell lysate, (3) cytosolic fraction lysate, (4) membrane fraction lysate and (5) nuclear fraction lysate. Membrane was blotted with a Plasma Membrane Fractionation WB cocktail <u>ab140365</u> containing anti-Sodium Potassium ATPase antibody (110 kDa), anti-GAPDH antibody (37 kDa) and anti-Histone H3 (di methyl K9) antibody.

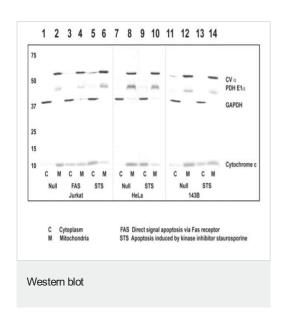


Western blot of cytoplasmic, mitchondrial and nuclear fractions

Cytosolic (C), mitochondrial (M) and nuclear (N) fractions of HepG2 cells were prepared as described in the Protocol. Fractions were analyzed by Western blotting using Abcam's ApoTrack™

Cytochrome c Apoptosis WB Antibody Cocktail (ab110415)

containing antibodies against mitochondrial matrix (pyruvate dehydrogenase subunit E1α, PDH E1α), mitochondrial inner membrane (F1-ATPase α), mitochondrial intermembrane space (cytochrome c) and cytosolic (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) markers as well as with antibodies against additional mitochondrial matrix (Hsp70) and nuclear (poly (ADP-ribose) polymerase, PARP and SP1) markers, followed by appropriate HRP-conjugated goat secondary antibodies and ECL detection.



In this experiment, apoptosis was induced in Jurkat, HeLa and 143B osteosarcoma cells by treatment with staurosporine or in Jurkat cells by FAS. Mitochondrial and cytoplasmic fractions were isolated (using ab109719) and probed using ab110415. As is clear from the gels, cytochrome c has translocated partially in FAS-induced cells and STS-treated osteosarcoma cells, and almost completely in STS-treated Jurkat and HeLa cells. The three control targets allow for verification of the "cleanness" of the cell fractionation.

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