abcam

Product datasheet

Membrane Fractionation Kit ab139409

3 References 5 Images

Overview

Product name	Membrane Fractionation Kit
Sample type	Cell culture extracts, Adherent cells, Suspension cells, Cell Lysate
Product overview	ab139409 provides the method and reagents for rapid separation of cytosolic, membrane and nuclear fractions from a wide variety of cell culture lines and species of origin. The kit is based on a sequential detergent-extraction of cytosolic and membrane proteins into the extracellular buffer using proprietary detergents. This method circumvents the need for time consuming and inefficient cell mechanical disruption and differential centrifugation steps. ab139409 fractionates the cells into cytosol-containing, membrane-containing and nuclei-containing fractions. These fractions are referred throughout the protocol as cytosolic, membrane and nuclear fractions. In the first step, the plasma membrane is selectively permeabilized with Detergent I. The cytosol-containing fraction is separated from the remainder cellular material containing membranes and nuclei by a simple centrifugation step. In the second step, membrane proteins are then extracted with Detergent II and separated from the nuclei-containing fraction by a second centrifugation step. The three distinct fractions generated can be run by gel electrophoresis and analyzed by Western Blot or run on high-performance liquid chromatography and analyzed by mass spectrometry.
Tested applications	**Suitable for:** Other

Properties

Storage instructions	Please refer to protocols.

Components	
5X SDS Sample Buffer	1 x 10ml
Detergent II	1 x 1ml
2X Buffer A	1 x 175ml

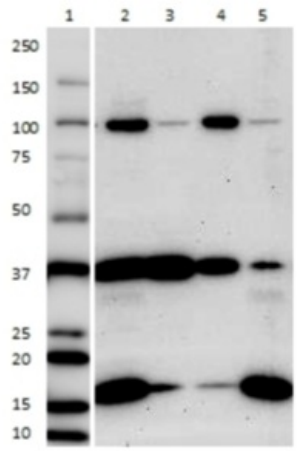
Applications

The Abpromise guarantee	Our Abpromise guarantee covers the use of ab139409 in the following tested applications.

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

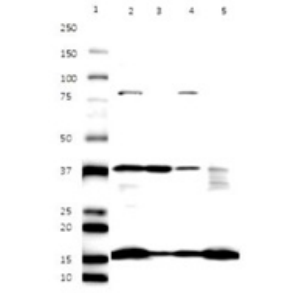
Application	Abreviews	Notes
Other		Use at an assay dependent concentration. Cell fractionation

Images



Analysis of Fractions by Western Blot with a Plasma membrane antibody cocktail

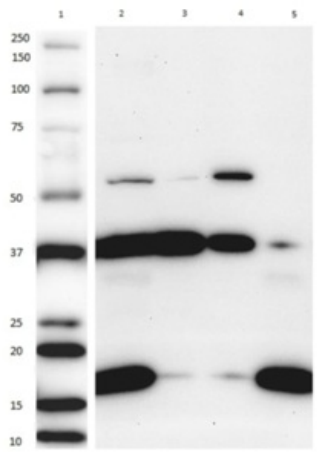
Developed using ECL
Performed under reducing conditions
Exposure time: 5 mins
Blocking and antibody incubation steps were done in 5% milk, 20mM Tris-HCl, 0.1% TWEEN-20
Lane 1: Marker
Lane 2: HeLa Whole Cell Lysate - 20 µL
Lane 3: HeLa Cytosolic Fraction Lysate - 20 µL
Lane 4: HeLa Membrane Fraction Lysate - 20 µL
Lane 5: HeLa Nuclear Fraction Lysate – 20 µL
All Lanes:
Anti-Sodium ATPase (ab7671) antibody – Plasma Membrane Marker – 1 µg/ml
Anti-GAPDH (ab8245) antibody – Cytosolic Marker – 0.0125 µg/ml
Anti-Histone H3 (di methyl k9) (ab1220) antibody – Nuclear Marker – 1 µg/ml
Secondary: Goat polyclonal to Mouse IgG (ab6789) – H&L – Pre-Adsorbed (HRP) at 1/10000 dilution
Predicted Sodium Potassium ATPase band size: 112 kDa, Observed band size: 100 kDa
Predicted GAPDH band size: 37 kDa, Observed band size: 37 kDa
Predicted Histone H3 (di methyl k9) band size: 17 kDa, Observed band size: 17 kDa



Analysis of Fractions by Western Blot with ER antibody cocktail

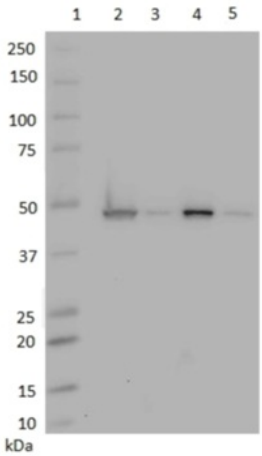
Performed under reducing conditions, Developed using ECL
Exposure time: 5 mins
Lane 1: Marker
Lane 2: HeLa Whole Cell Lysate 20µL
Lane 3: HeLa Cytosolic Fraction Lysate 20µL
Lane 4: HeLa Membrane Fraction Lysate 20µL
Lane 5: HeLa Nuclear Fraction Lysate 20µL
All Lanes:
Anti- GRP78 (ab108613) antibody – Endoplasmic Reticulum Marker 1/1000
Anti- GAPDH (ab8245) antibody – Cytosolic Marker 0.0125 µg/ml
Anti-Histone H3 (di methyl K9) band size: (ab1220) antibody –

Nuclear Marker 1 µg/ml
Secondary: Goat polyclonal to Mouse IgG (ab6789) – H&L – Pre-Absorbed (HRP) at 1/10000 dilution and Goat polyclonal to Rabbit IgG (ab6721) – H&L – Pre-Absorbed (HRP) at 1/10000 dilution
Predicted GRP78 band size: 78 kDa, Observed 78 kDa
Predicted GAPDH band size: 37 kDa, Observed 37 kDa
Predicted Histone H3 (di methyl K9) band size: 17 kDa, Observed 17 kDa



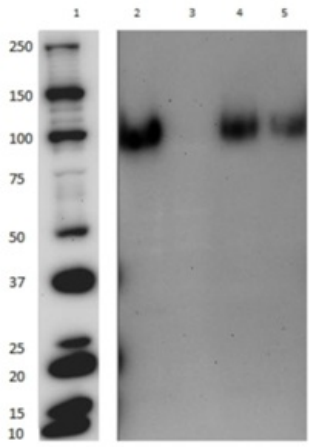
Analysis of Fractions by Western Blot with a Mitochondria antibody cocktail

Developed using the ECL
Performed under reducing conditions
Exposure time: 5 mins
All blocking and antibody incubation steps were done in 5% milk, 20 mM Tris-HCl, 0.1% TWEEN-20
Lane 1: Marker
Lane 2: HeLa Whole Cell Lysate - 20 µL
Lane 3: HeLa Cytosolic Fraction Lysate - 20 µL
Lane 4: HeLa Membrane Fraction Lysate - 20 µL
Lane 5: HeLa Nuclear Fraction Lysate - 20 µL
All Lanes:
Anti- ATP5A (ab110273) antibody – Mitochondrial Membrane Marker – 0.0125 µg/ml
Anti- GAPDH (ab8245) antibody – Cytosolic Membrane Marker – 0.0125 µg/ml
Anti-Histone 3 (ab1220) antibody – Nuclear Membrane Marker – 1 µg/ml
Secondary: Goat polyclonal to Mouse IgG (ab6789) – H&L – Pre-Adsorbed (HRP) at 1/10000 dilution
Predicted ATP5a band size: 60 kDa, Observed ATP5a band size: 55kDa
Predicted GAPDH band size: 37 kDa, Observed GAPDH band size: 37 kDa
Predicted Histone 3 band size: 17 kDa, Observed Histone 3 band size: 17 kDa



Analysis of Fractions by Western Blot with an Endoplasmic Reticulum Marker

Developed using the ECL technique
Performed under reducing conditions
Exposure time: 5 minutes
All blocking and antibody incubation steps were done in 5% milk, 20mM Tris-HCL, 0.1% TWEEN-20
Sample Preparation HeLa cell lysate was prepared using the Cell Fractionation Kit ab109719. Each fraction is resuspended in the same volume as the original lysate, i.e., if all of the Histone H3 goes to one fraction, the band intensity should be comparable to the whole cell lysate
Lane 1: Marker
Lane 2: HeLa Whole Cell Lysate 20 µL
Lane 3: HeLa Cytosolic Fraction Lysate 20 µL
Lane 4: HeLa Membrane Fraction Lysate 20 µL
Lane 5: HeLa Nuclear Fraction Lysate 20 µL
All lanes: Anti-PDI antibody (ab5484) -Endoplasmic Reticulum Membrane Marker at 1/1000 dilution.
Secondary: Goat polyclonal to Mouse IgG-H&L- Pre-Adsorbed (HRP) at 1/10000 dilution
Predicted GM130 band size: 59 kDa Observed band size: 45 kDa



Analysis of Fractions by Western Blot with a Lysosome Marker

Developed using the ECL technique.
Performed under reducing conditions.
Exposure time: 5 minutes
All blocking and antibody incubation steps were done in 5% milk, 20mM Tris-HCL, 0.1% TWEEN-20
Sample Preparation: HeLa cell lysate was prepared using the Membrane fractionation kit. Each fraction was resuspended in the same volume as the original lysate.
Lane 1: Marker
Lane 2: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate 20 µL
Lane 3: HeLa (Human epithelial carcinoma cell line) Cytosolic Fraction Lysate 20 µL
Lane 4: HeLa (Human epithelial carcinoma cell line) Membrane

Fraction Lysate 20 µL

Lane 5: HeLa (Human epithelial carcinoma cell line) Nuclear Fraction Lysate 20 µl

All lanes: Anti-LAMP2 antibody (ab25631)-Lysosome Membrane Marker at 1/5000 dilution

Secondary: Goat polyclonal to Mouse IgG-H&L- Pre-Adsorbed (HRP) at 1/10000 dilution

Predicted band size: 110 kDa. Observed band size: 110 kDa

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