abcam

Product datasheet

Plasma Membrane Fraction Western Blot Cocktail ab139413

2 References 3 Images

Overview

Product name	Plasma Membrane Fraction Western Blot Cocktail
Sample type	Tissue Extracts, Cell Lysate, Tissue Homogenate, Nuclear Extracts
Assay type	Indirect
Species reactivity	**Reacts with:** Mouse, Rat, Human
Product overview	ab139413 contains 3 mouse monoclonal antibodies each targeting a specific organelle marker. The presence of plasma membrane is determined by Anti-Sodium Potassium ATPase antibody; cytosol by Anti-GAPDH; and nucleus by Anti-Histone H3 (di methyl K9). This cocktail is suitable for determining the purity of organelle isolates prior to further characterization. This product is particularly valuable to researchers working in organelle proteomics. Mass spectrometry is frequently used in this field to determine the protein content of targeted organelle isolates. These isolates are obtained using differential centrifugation, density gradient fractionation, biochemical enrichment, or affinity purification. Unfortunately, the various methods of purification available for organelle isolation are imperfect and leave behind contaminants from undesired regions of the cell. These contaminants are inevitable, but being aware of which contaminants are present is crucial for analysis of mass spectrometry results. The high sensitivity and species cross reactivity of the antibodies in this cocktail will quickly and easily reveal impurities caused by imperfect sample preparation.
Tested applications	**Suitable for:** WB

Properties

Storage instructions	Store at +4°C. Please refer to protocols.

Components	200 µl
250X Plasma Membrane Fraction WB Cocktail	1 x 200µl

Cellular localization	Sodium Potassium ATPase: Cell membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV. GAPDH: Cytoplasm > cytosol. Nucleus. Cytoplasm > perinuclear region. Membrane. Translocates to the nucleus following S-nitrosylation and interaction with SIAH1, which contains a nuclear localization signal (By similarity). Postnuclear

and Perinuclear regions. Histone H3: Nucleus. Chromosome.

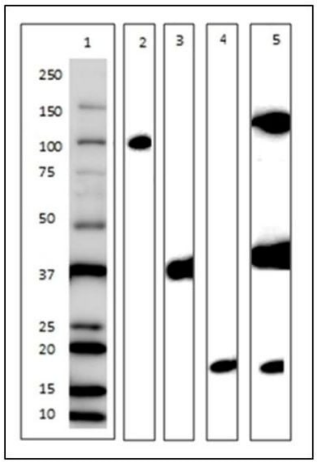
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab139413 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
WB		1/250.

Images



Western Blot for Plasma Membrane Antibody Cocktail – Component Separation

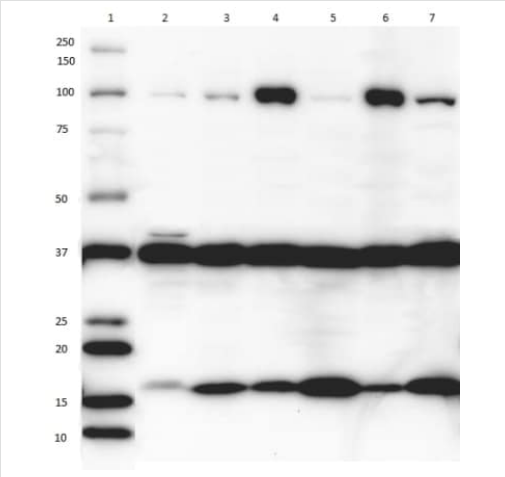
Developed using the ECL technique; Performed under reducing conditions; Exposure time: 5 mins; All blocking and antibody incubation steps were done in 5% milk, 20 mM Tris-HCl, 0.1% TWEEN-20

Lane 1: Marker
Lanes 2-5: HeLa Whole Cell Lysate – 20 µg

Primary antibodies:
Lane 1: none
Lane 2: Anti- Sodium ATPase antibody – Plasma Membrane Marker
Lane 3: Anti- GAPDH antibody – Cytosolic Membrane Marker
Lane 4: Anti-Histone 3 antibody – Nuclear Membrane Marker
Lane 5: Assembled Plasma Membrane Antibody Cocktail

Secondary: ab131368 at 1/1000 dilution

Predicted Sodium Potassium ATPase band size: 112 kDa
Observed Sodium Potassium ATPase band size: 100 kDa
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 kD



Plasma Membrane Western Blot Cocktail Cross Reactivity

Developed using ECL technique under reducing conditions; exposure time 3 mins; blocking and antibody incubation steps done in 5% milk/TBST

Lanes:

1: Marker

2: Human heart homogenate Tissue Lysate – 20 µg

3: HeLa Cell Lysate – 20 µg

4: Mouse heart homogenate Tissue Lysate - 20 µg

5: NIH3T3 Cell Lysate – 20 µg

6: Rat heart homogenate Tissue Lysate – 20 µg

7: H9C2 cell lysate – 20 µg

All Lanes:

Anti-Sodium ATPase antibody – Plasma Membrane Marker

Anti-GAPDH antibody – Cytosolic Marker

Anti-Histone H3 (di methyl k9) antibody – Nuclear Marker

Secondary: ab131368 at 1/1000 dilution

Predicted Sodium Potassium ATPase band size: 112 kDa

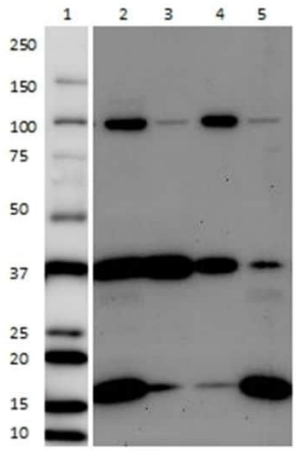
Observed Sodium Potassium ATPase band size: 100 kDa

Predicted GAPDH band size: 37 kDa

Observed GAPDH band size: 37 kDa

Predicted Histone H3 (di methyl k9) band size: 17 kDa

Observed Histone H3 (di methyl k9 band size: 17 kDa



WB analysis of HeLa cell fractions.

Developed using ECL technique, reducing conditions, exposured 5 mins

Blocking and antibody incubations done in 5% milk, 20 mM Tris-HCl, 0.1% TWEEN-20

Lanes:

1: Marker

2: HeLa Whole Cell Lysate - 20 µl

3: HeLa Cytosolic Fraction Lysate - 20 µl

4: HeLa Membrane Fraction Lysate - 20 µl

5: HeLa Nuclear Fraction Lysate - 20 µl

All Lanes:

Anti-Sodium ATPase antibody – Plasma Membrane Marker

Anti-GAPDH antibody – Cytosolic Membrane Marker

Anti-Histone 3 antibody – Nuclear Membrane Marker

Secondary:

Goat polyclonal to Mouse IgG (ab6789) – H&L (HRP)

Predicted Sodium Potassium ATPase band size: 112 kDa

Observed Sodium Potassium ATPase band size: 100 kDa

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

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