

Organelle Detection Western Blot Cocktail ab133989

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Overview

Product name	Organelle Detection Western Blot Cocktail
Sample type	Cell culture extracts, Adherent cells, Suspension cells, Tissue Extracts, Cell Lysate, Tissue Homogenate, Nuclear Extracts
Assay type	Quantitative
Species reactivity	Reacts with: Mouse, Rat, Human
Product overview	<p>ab133989 contains 4 mAbs each targeting a specific organelle marker. The presence of plasma membrane is determined by Anti-Sodium Potassium ATPase antibody; mitochondrion by Anti-ATP5A 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.</p> <p>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.</p>
Tested applications	Suitable for: WB

Properties

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

Components	200 µl
Organelle Detection Western Blot 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. ATP5A: Mitochondrion inner membrane. Peripheral membrane protein. 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

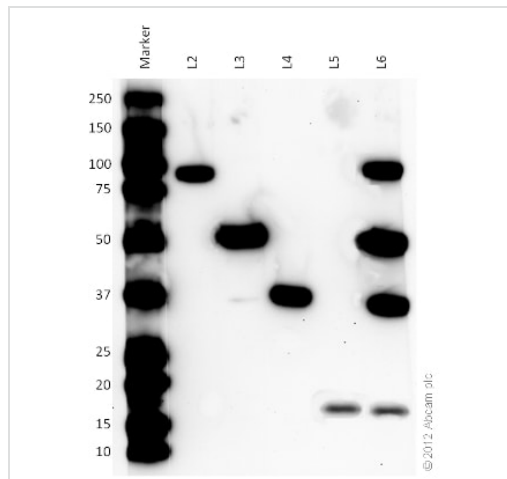
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab133989 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		Use at an assay dependent concentration. The antibody cocktail should be diluted 250 X for western blotting.

Images



Western blot - Organelle Detection Western Blot
Cocktail (ab133989) - Component Separation

All blocking and antibody incubation steps were done in 5% milk, 20 mM Tris-HCl, 0.1% TWEEN-20.

Lane 2-6 : Mouse heart homogenate Whole Tissue Lysate 10 µg
Primary antibody:

Lane 2 : Anti-Sodium Potassium ATPase antibody – Plasma Membrane Marker

Lane 3 : Anti-ATP5A antibody – Mitochondrial Marker

Lane 4 : Anti-GAPDH antibody – Cytosolic Marker

Lane 5 : Anti-Histone H3 (di methyl K9) antibody – Nuclear Marker

Lane 6 : Assembled Organelle Detection Cocktail

Secondary: **ab131368** at 1/1000 dilution.

Predicted Sodium Potassium ATPase band size : 113 kDa

Observed band size : 85 kDa

Predicted ATP5A band size : 60 kDa

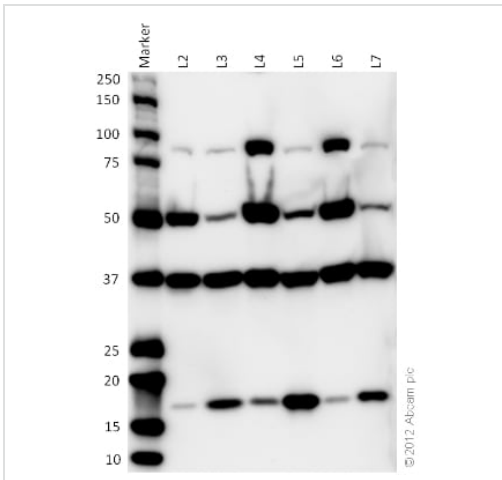
Observed ATP5A band size : 52 kDa

Predicted sample band size : 36 kDa

Observed band size : 36 kDa

Predicted sample band size : 15.5 kDa

Observed band size : 17 kDa



Western blot - Organelle Detection Western Blot
Cocktail (ab133989) - Cross reactivity

All blocking and antibody incubation steps were done in 5% milk, 20 mM Tris-HCl, 0.1% TWEEN-20.

All lanes :

Anti-Sodium Potassium ATPase antibody – Plasma Membrane

Marker

Anti-ATP5A antibody – Mitochondrial Marker

Anti-GAPDH antibody – Cytosolic Marker

Anti-Histone H3 (di methyl K9) antibody – Nuclear Marker

Lane 1 : Marker

Lane 2 : Human heart homogenate Whole Tissue Lysate - 20 µg

Lane 3 : HeLa Whole Cell Lysate - 20 µg

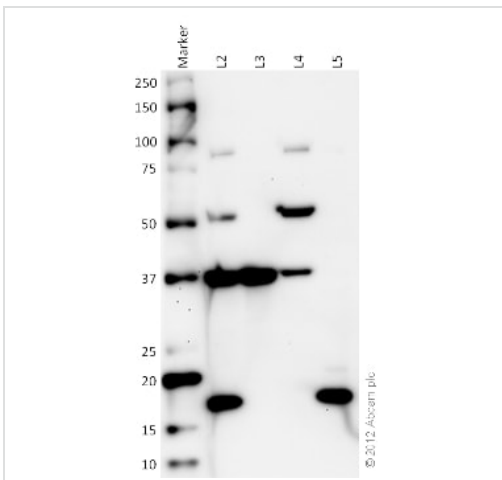
Lane 4 : Mouse heart homogenate Whole Tissue Lysate - 20 µg

Lane 5 : NIH-3T3 Whole Cell Lysate - 20 µg

Lane 6 : Rat heart homogenate Whole Tissue Lysate - 20 µg

Lane 7 : H9C2 Whole Cell Lysate - 20 µg

Secondary: **ab131368** at 1/1000 dilution.



Western blot - Organelle Detection Western Blot
Cocktail (ab133989) - Cell Fractions

HeLa cell lysate was prepared using the Cell Fractionation Kit

ab109719. All blocking and antibody incubation steps were done in 5% milk, 20 mM Tris-HCl, 0.1% TWEEN-20.

All lanes :

Anti-Sodium Potassium ATPase antibody – Plasma Membrane

Marker

Anti-ATP5A antibody – Mitochondrial Marker

Anti-GAPDH antibody – Cytosolic Marker

Anti-Histone H3 (di methyl K9) antibody – Nuclear Marker

Lane 1 : Marker

Lane 2 : HeLa Whole Cell Lysate

Lane 3 : HeLa Cytosolic Fraction Lysate

Lane 4 : HeLa Mitochondrial Fraction Lysate

Lane 5 : HeLa Nuclear Fraction Lysate

Secondary

Goat polyclonal to Mouse IgG – H&L – Pre-Adsorbed (HRP) at 1/10000.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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