

Mitochondria Fraction Western Blot Cocktail ab139416

[3 References](#) [3 Images](#)

Overview

Product name	Mitochondria Fraction Western Blot Cocktail
Sample type	Cell culture extracts, Adherent cells, Suspension cells, Tissue Extracts, Cell Lysate, Tissue Homogenate
Species reactivity	Reacts with: Mouse, Rat, Human
Product overview	Mitochondria Fraction Western Blot Cocktail (ab139416) contains 3 Mouse mAbs each targeting a specific organelle marker. The presence of mitochondria is determined by Anti-ATP5A ; 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 Mitochondria Fraction WB Cocktail	1 x 200µl

Cellular localization ATP5A: Mitochondrion inner membrane. Cell membrane; Peripheral membrane protein; Extracellular side. GAPDH: Cytoplasm; cytosol. Nucleus. Cytoplasm; perinuclear region. Membrane. Cytoplasm; cytoskeleton Histone 3: Nucleus. Chromosome.

Applications

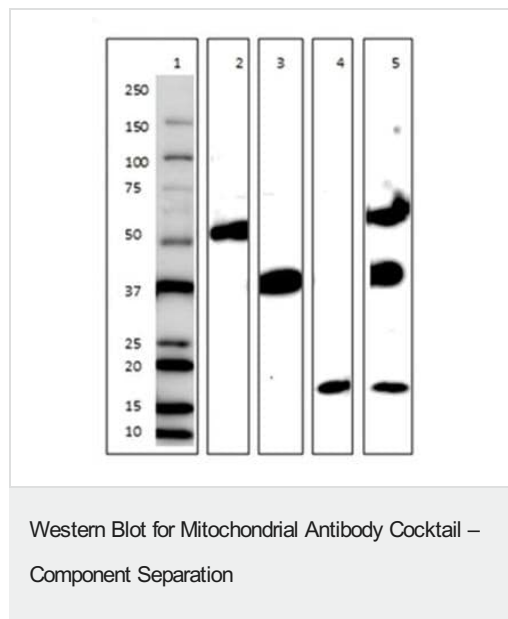
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab139416 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. Suggested working concentration: 1/250 dilution

Images



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

Sample 1: Marker

Samples 2-5: MHH (Mouse heart homogenate) Whole Tissue Lysate – 20 µg

Primary:

Lane 1: none

Lane 2: Anti- ATP5A antibody – Mitochondrial Membrane Marker

Lane 3: Anti- GAPDH antibody – Cytosolic Membrane Marker

Lane 4: Anti-Histone H3 (di methyl K9) antibody – Nuclear Membrane Marker

Lane 5: Assembled Mitochondrial Membrane Antibody Cocktail

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

Predicted ATP5a band size: 60 kDa

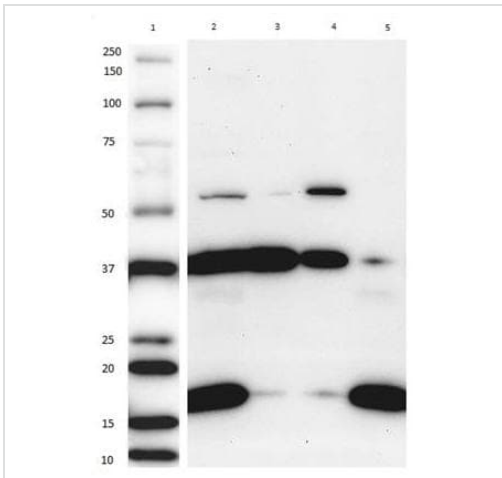
Observed ATP5a band size: 55 kDa

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



Western Blot for Mitochondrial Antibody Cocktail –
HeLa Fractions

Sample Preparation: HeLa cell lysate prepared using the Membrane Fractionation Kit ([ab139409](#)); Developed using the ECL technique under reducing conditions; Exposure time: 5 mins; Blocking and antibody incubation steps done in 5% milk, 20 mM Tris-HCl, 0.1% TWEEN-20

Lane 1: Marker

Lane 2 : HeLa Whole Cell Lysate - 20 μ L

Lane 3 : HeLa Cytosolic Fraction Lysate - 20 μ L

Lane 4 : HeLa Membrane Fraction Lysate - 20 μ L

Lane 5 : HeLa Nuclear Fraction Lysate - 20 μ L

All Lanes:

Anti- ATP5A antibody – Mitochondrial Marker – 1/250 dilution

Anti- GAPDH antibody – Cytosolic Marker – 1/250 dilution

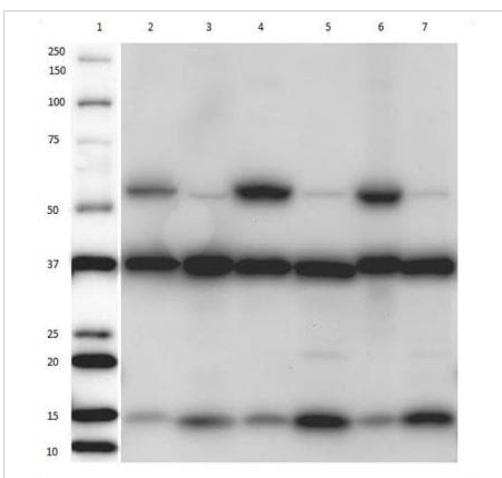
Anti-Histone H3 (di methyl K9) antibody – Nuclear Marker – 1/250 dilution

Secondary: Goat polyclonal to Mouse IgG ([ab6789](#)) – H&L – Pre-Absorbed (HRP) at 1/10000 dilution

Observed ATP5a band size: 55 kDa

Observed GAPDH band size: 37 kDa

Observed Histone H3 (di methyl K9) band size: 17 kDa



Western Blot for Mitochondrial Membrane Antibody
Cocktail – Cross Reactivity

Developed using the ECL technique; Performed under reducing conditions; Exposure time: 3 mins; All blocking and antibody incubation steps done in 5% milk, 20 mM Tris-HCl, 0.1% TWEEN-20

Sample 1: Marker

Sample 2: HHH Whole Tissue Lysate - 20 μ g

Sample 3: Hela Whole Cell Lysate – 20 μ g

Sample 4: MHH Whole Tissue Lysate - 20 μ g

Sample 5: NIH3T3 Whole Cell Lysate – 20 μ g

Sample 6: RHH Whole Tissue Lysate – 20 μ g

Sample 7: H9C2 Whole Cell Lysate – 20 μ g

All Lanes:

Anti- ATP5A antibody – Mitochondrial Marker – 1/250 dilution

Anti- GAPDH antibody – Cytosolic Marker – 1/250 dilution
Anti-Histone H3 (di methyl K9) antibody – Nuclear Marker – 1/250 dilution

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

Observed ATP5A band size: 60 kDa

Observed GAPDH band size: 37 kDa

Observed Histone H3 (di methyl K9) band size: 15 kDa

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