abcam

Product datasheet

Mitochondria Fraction Western Blot Cocktail ab139416

3 References 画像数 3

製品の概要

製品名 Mitochondria Fraction Western Blot Cocktail

サンプルの種類 Cell culture extracts, Adherent cells, Suspension cells, Tissue Extracts, Cell Lysate, Tissue

Homogenate

種交差性 交差種: Mouse, Rat, Human

製品の概要 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.

アプリケーション 適用あり: WB

製品の特性

保存方法 Store at +4°C. Please refer to protocols.

内容	200 μΙ
250X Mitochondria Fraction WB Cocktail	1 x 200µl

細胞内局在

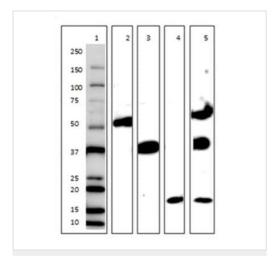
ATP5A: Mitochondrion inner membrane. Cell membrane; Peripheral membrane protein; Extracellular side. GAPDH: Cytoplasm; cytosol. Nucleus. Cytoplasm; perinuclear region. Membrane. Cytoplasm; cytoskeleton Histone 3: Nucleus. Chromosome.

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アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Suggested working concentration: 1/250 dilution

画像



Western Blot for Mitochondrial 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

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

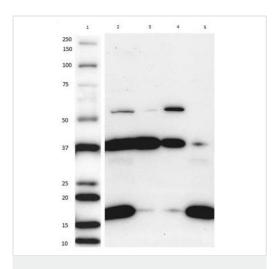
Lane 5: Assembled Mitochondrial Membrane Antibody Cocktail

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-HCI. 0.1% TWEEN-20

Lane 1: Marker

Lane 2: HeLa Whole Cell Lysate - 20 µL

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 $\lg G \ (\underline{ab6789}) - H\&L - Pre-Absorbed \ (HRP)$ 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

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-HCI, 0.1% TWEEN-

20

250 150 100 75 50 37 25 20

Western Blot for Mitochondrial Membrane Antibody Cocktail – Cross Reactivity

Sample 1: Marker

Sample 2: HHH Whole Tissue Lysate - 20 μg

Sample 3: Hela Whole Cell Lysate - 20 µg

Sample 4: MHH Whole Tissue Lysate - 20 µg

Sample 5: NIH3T3 Whole Cell Lysate - 20 µg

Sample 6: RHH Whole Tissue Lysate – 20 µg

Sample 7: H9C2 Whole Cell Lysate - 20 µg

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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