OXPHOS Cocktail Antibody Sampler Kit II

HAK21116

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
ATP5A1 [ET1703-53]	20µl	WB,IF-Cell,IF-Tissue,IHC-P	H,M,R	60 kDa
UQCRC2 [HA721872]	20µl	WB,IF-Cell,IHC-P,FC	H,M,R	48 kDa
SDHA [ET1703-40]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,FC	H,M,R,Z	73 kDa
SDHB [HA722901]	20µl	WB,IHC-P,IP,IF-Cell	H,M,R	32 kDa
MTCO2 [ET1610-72]	20µl	WB,IF-Cell,IF-Tissue,IHC-P	H	26 kDa
MTCO1 [HA722838]	20µl	WB,IF-Cell,IHC-P,FC,IF-Tissue	H,M,R	57 kDa
NDUFV2 [HA721869]	20µl	WB,IHC-P,IF-Cell	H,M,R	27 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100µl	WB,ELISA,IHC-P	Rab	

Description: The OXPHOS Cocktail Antibody Sampler kit provides an economical means to investigate the relative levels of five OXPHOS complexes in mitochondrial protein samples from humans. The OXPHOS Cocktail Antibody Sampler kit contains enough primary and secondary antibodies to perform two Western blot experiments.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Background OXPHOS (oxidative phosphorylation) transfer electrons to molecular oxygen through the mitochondrial respiratory chain, which involves four protein complexes. Complexes I, II, III, and IV (CI, CII, CIII, and CIV) and two mobile electron carriers: Coenzyme Q (CoQ) and cytochrome c. The respiratory chain produces a transmembrane proton gradient guided by complex V (also known as ATP synthase, CV) to synthesize ATP.

Database links: UniProt ID: P25705, Q03265, P15999, P22695, Q9DB77, P32551, P31040, Q8K2B3, Q920L2, P21912, Q9CQA3, P21913, P00403, P00395, P00397, P05503, P19404, Q9D6J6, P19234

Images

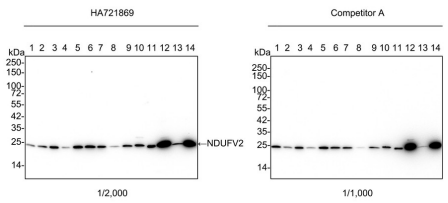

Fig1: Western blot analysis of NDUFV2 on different lysates with Rabbit anti-NDUFV2 antibody (HA721869) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: A549 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: Jurkat cell lysate (20 µg/Lane)
Lane 4: Ramos cell lysate (20 µg/Lane)
Lane 5: Raji cell lysate (20 µg/Lane)
Lane 6: K-562 cell lysate (20 µg/Lane)
Lane 7: A431 cell lysate (20 µg/Lane)
Lane 8: NIH/3T3 cell lysate (20 µg/Lane)
Lane 9: RAW264.7 cell lysate (20 µg/Lane)
Lane 10: PC-12 cell lysate (20 µg/Lane)
Lane 11: Mouse heart tissue lysate (40 µg/Lane)
Lane 12: Rat heart tissue lysate (40 µg/Lane)
Lane 13: Human kidney tissue lysate (40 µg/Lane)
Lane 14: Mouse kidney tissue lysate (40 µg/Lane)

Predicted band size: 27 kDa
Observed band size: 24 kDa

Exposure time: 1 minute 59 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721869) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

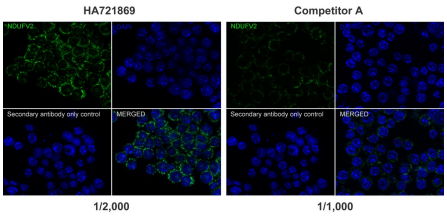

Fig2: Immunocytochemistry analysis of RAW264.7 cells labeling NDUFV2 with Rabbit anti-NDUFV2 antibody (HA721869) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-NDUFV2 antibody (HA721869) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

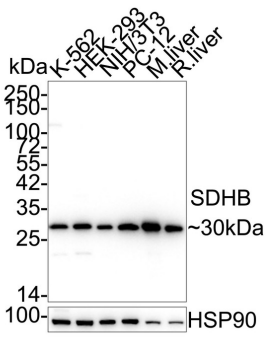

Fig3: Western blot analysis of SDHB on different lysates with Rabbit anti-SDHB antibody (HA722901) at 1/2,000 dilution.

Lane 1: K-562 cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: PC-12 cell lysate
Lane 5: Mouse liver tissue lysate
Lane 6: Rat liver tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 32 kDa
Observed band size: 30 kDa

Exposure time: 4 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722901) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

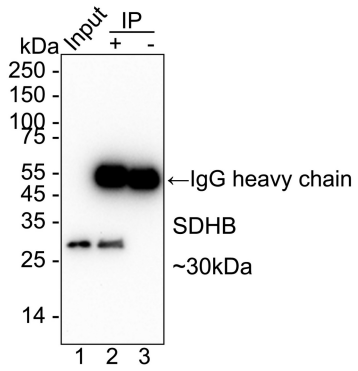

Fig4: SDHB was immunoprecipitated from 0.2 mg HEK-293 cell lysate with HA722901 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA722901 at 1/1,000 dilution. Mouse anti Rabbit IgG heavy chain (Fc) secondary antibody (M1003-7) at 1/100,000 dilution was used for 1 hour at room temperature.

Lane 1: HEK-293 cell lysate (input)
Lane 2: HA722901 IP in HEK-293 cell lysate
Lane 3: Rabbit IgG instead of HA722901 in HEK-293 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 40 seconds; ECL: K1801

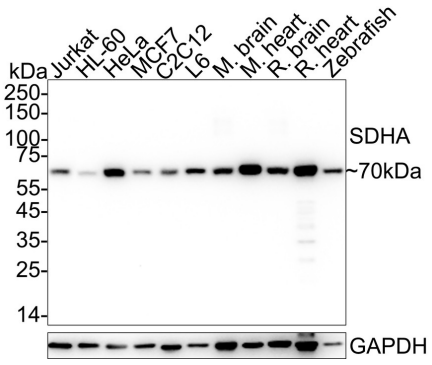

Fig5: Western blot analysis of SDHA on different lysates with Rabbit anti-SDHA antibody (ET1703-40) at 1/1,000 dilution.

Lane 1: Jurkat cell lysate (15 µg/Lane)
Lane 2: HL-60 cell lysate (15 µg/Lane)
Lane 3: HeLa cell lysate (15 µg/Lane)
Lane 4: MCF7 cell lysate (15 µg/Lane)
Lane 5: C2C12 cell lysate (15 µg/Lane)
Lane 6: L6 cell lysate (15 µg/Lane)
Lane 7: Mouse brain tissue lysate (30 µg/Lane)
Lane 8: Mouse heart tissue lysate (30 µg/Lane)
Lane 9: Rat brain tissue lysate (30 µg/Lane)
Lane 10: Rat heart tissue lysate (30 µg/Lane)
Lane 11: Zebrafish tissue lysate (30 µg/Lane)

Predicted band size: 73 kDa
Observed band size: 70 kDa

Exposure time: 5 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1703-40) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

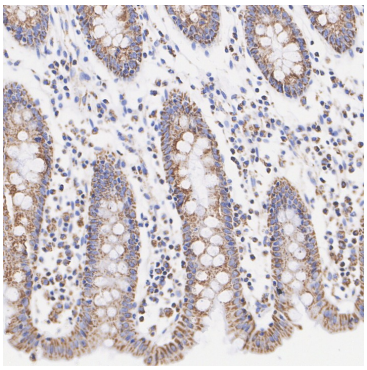

Fig6: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-MTCO1 antibody (HA722838) at 1/10,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722838) at 1/10,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. 1. Vercellino I, Sazanov LA. The assembly, regulation and function of the mitochondrial respiratory chain. Nat Rev Mol Cell Biol. 2022 Feb;23(2):141-161.
2. 2. Nolfi-Donegan D, Braganza A, Shiva S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. Redox Biol. 2020 Oct;37:101674.