abcam

Product datasheet

Anti-Ndufs4 antibody [EP7832] ab137064

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Ndufs4 antibody [EP7832]
Description	Rabbit monoclonal [EP7832] to Ndufs4
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Sheep, Goat, Cat, Dog, Pig, Common marmoset **Does not react with:** Eisenia fetida
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	293T cell lysates, fetal brain and fetal stomach tissue lysates; Human brain and Human stomach tissues Mouse heat lysate, rat heart lysate, mouse kidney, HeLa.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EP7832
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab137064 in the following tested applications.

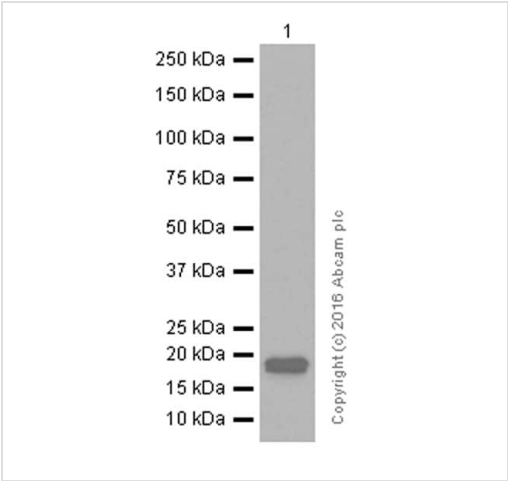
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/60. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurifed use at 1/100 - 1/1000 dilution.
WB	★★★★★ (1)	1/500 - 1/10000. Detects a band of approximately 18 kDa (predicted molecular weight: 20 kDa).
IHC-P	★★★★★ (10)	1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★☆ (2)	1/500. **For unpurified use at 1/50 - 1/100.**
IP		1/10 - 1/100.

Target

Function	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed not to be involved in catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone.
Involvement in disease	Defects in NDUFS4 are a cause of mitochondrial complex I deficiency (MT-C1D) [MIM:252010]. A disorder of the mitochondrial respiratory chain that causes a wide range of clinical disorders, from lethal neonatal disease to adult-onset neurodegenerative disorders. Phenotypes include macrocephaly with progressive leukodystrophy, non-specific encephalopathy, cardiomyopathy, myopathy, liver disease, Leigh syndrome, Leber hereditary optic neuropathy, and some forms of Parkinson disease.
Sequence similarities	Belongs to the complex I NDUFS4 subunit family.
Cellular localization	Mitochondrion inner membrane.

Images



Western blot - Anti-Ndufs4 antibody [EP7832] (ab137064)

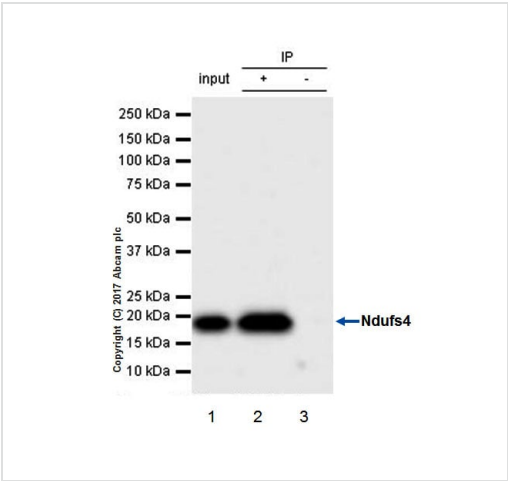
Anti-Ndufs4 antibody [EP7832] (ab137064) at 1/50000 dilution (purified) + Rat heart lysates at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 20 kDa

Blocking and diluting buffer: 5% NFDM/TBST

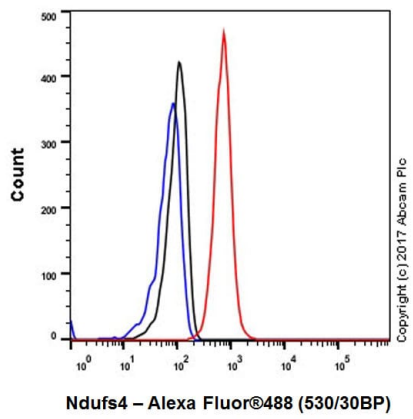


Immunoprecipitation - Anti-Ndufs4 antibody [EP7832] (ab137064)

ab137064 (purified) at 1:30 dilution (2µg) immunoprecipitating Ndufs4 in Rat heart lysate.

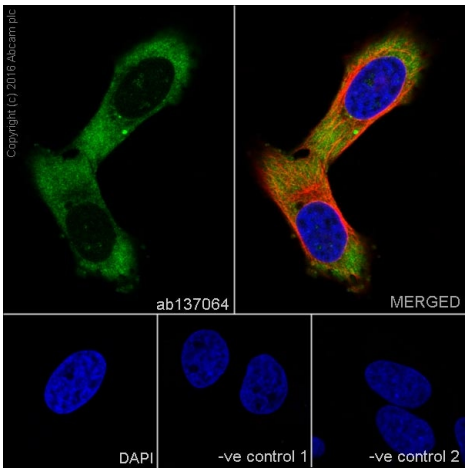
Lane 1 (input): Rat heart lysate, 10µg
Lane 2 (+): ab137064 & Rat heart lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab137064 in Rat heart lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.



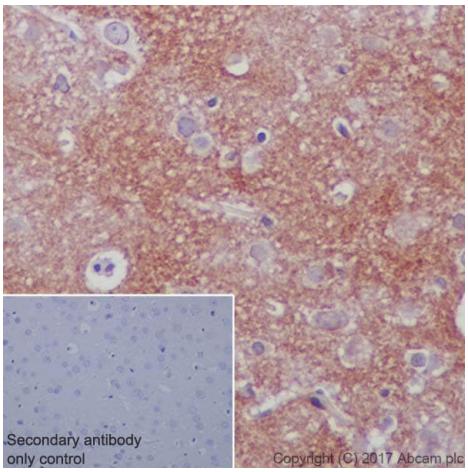
Flow Cytometry (Intracellular) - Anti-Ndufs4 antibody [EP7832] (ab137064)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Ndufs4 with purified ab137064 at 1/60 dilution (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



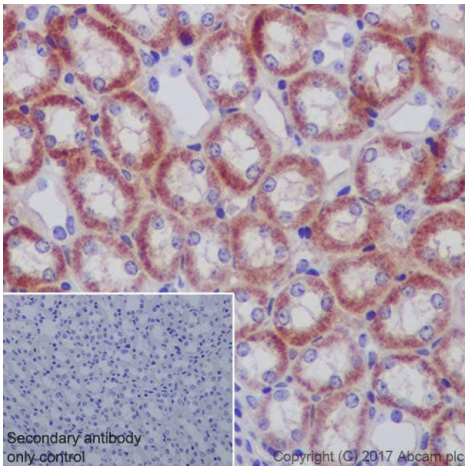
Immunocytochemistry/ Immunofluorescence - Anti-Ndufs4 antibody [EP7832] (ab137064)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Ndusf5 with Purified ab137064 at 1:500 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab7291** anti-Tubulin (mouse mAb) **ab150120** AlexaFluor ® 594 Goat anti-Mouse secondary (1:1000,2 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor ® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



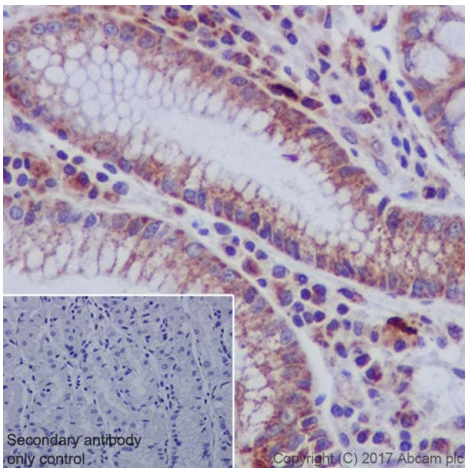
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ndufs4 antibody [EP7832] (ab137064)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat cerebrum tissue sections labeling Ndufs4 with Purified ab137064 at 1:50 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH 9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



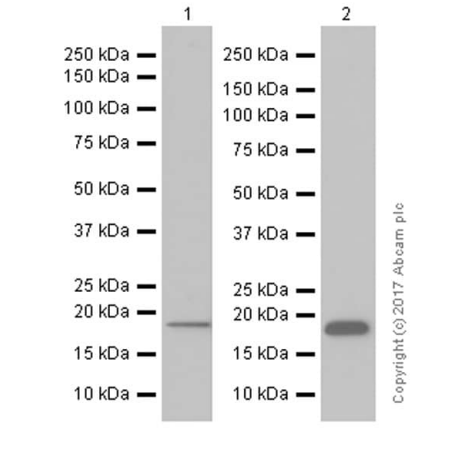
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ndufs4 antibody [EP7832] (ab137064)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling Ndufs4 with Purified ab137064 at 1:50 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH 9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ndufs4 antibody [EP7832] (ab137064)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human stomach tissue sections labeling Ndufs4 with Purified ab137064 at 1:50 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH 9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-Ndufs4 antibody [EP7832] (ab137064)

All lanes : Anti-Ndufs4 antibody [EP7832] (ab137064) at 1/2000 dilution (purified)

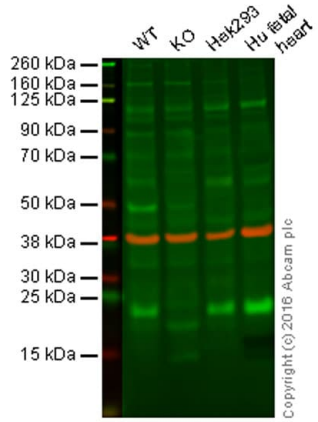
Lane 1 : Human fetal brain lysates
Lane 2 : Mouse heart lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 20 kDa
Observed band size: 18 kDa

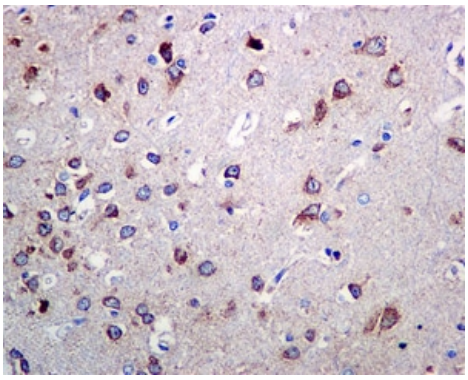
Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-Ndufs4 antibody [EP7832] (ab137064)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: Ndufs4 knockout HAP1 cell lysate (20 µg)
Lane 3: HEK293 cell lysate (20 µg)
Lane 4: Human fetal heart tissue lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab137064 observed at 23 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

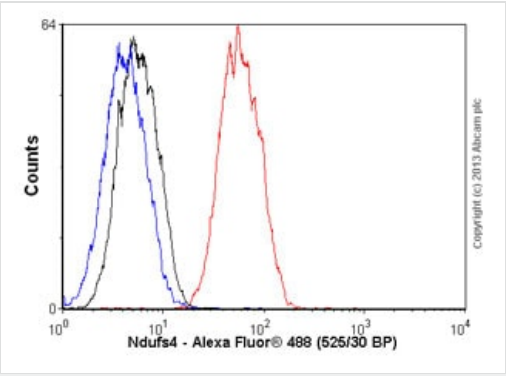
Unpurified ab137064 was shown to recognize Ndufs4 when Ndufs4 knockout samples were used, along with additional cross-reactive bands. Wild-type and Ndufs4 knockout samples were subjected to SDS-PAGE. ab137064 and **ab8245** (loading control to GAPDH) were diluted at 1/500 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ndufs4 antibody [EP7832] (ab137064)

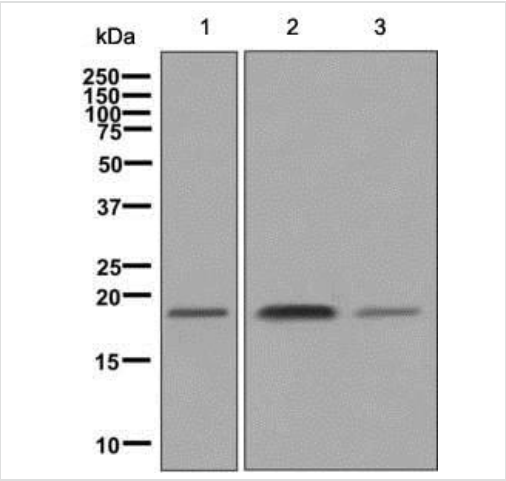
Immunohistochemical analysis of paraffin-embedded Human brain tissue labelling Ndufs4 with unpurified ab137064 at 1/50 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Ndufs4 antibody [EP7832] (ab137064)

Overlay histogram showing HepG2 cells stained withunpurified ab137064 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab137064, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HepG2 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



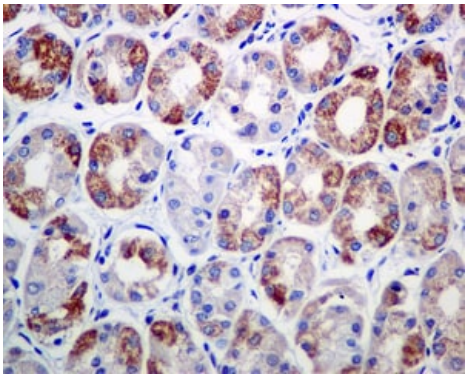
Western blot - Anti-Ndufs4 antibody [EP7832] (ab137064)

All lanes : Anti-Ndufs4 antibody [EP7832] (ab137064) at 1/1000 dilution (unpurified)

Lane 1 : 293T cell lysate
Lane 2 : Fetal brain tissue lysate
Lane 3 : Fetal kidney tissue lysate

Lysates/proteins at 10 µg per lane.

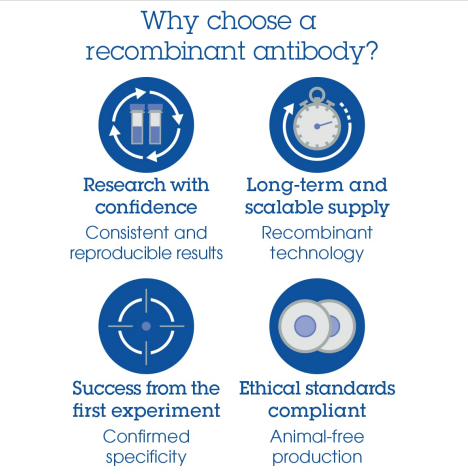
Predicted band size: 20 kDa
Observed band size: 18 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ndufs4 antibody [EP7832] (ab137064)

Immunohistochemical analysis of paraffin-embedded Human stomach tissue labelling Ndufs4 with unpurified ab137064 at 1/50 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Anti-Ndufs4 antibody [EP7832] (ab137064)

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