abcam

Product datasheet

Anti-NDUFS3 antibody [EPR12782] - C-terminal ab177471



Recombinant

RabMAb

* ★ ★ ★ ★ 2 Abreviews 7 References 10 Images

Overview

Product name Anti-NDUFS3 antibody [EPR12782] - C-terminal

Description Rabbit monoclonal [EPR12782] to NDUFS3 - C-terminal

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control HepG2, 293T, HL60 and fetal heart lysates. Human heart and kidney tissue. 293 cells.

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: 7.20

Preservative: 0.01% Sodium azide

Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture

supernatant

Purity Tissue culture supernatant

Clonality Monoclonal

1

Clone number

EPR12782

Isotype

lgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab177471 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)		1/200.
WB	**** <u>(1)</u>	1/1000 - 1/10000. Predicted molecular weight: 30 kDa.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★ <u>(1)</u>	1/50 - 1/100.
IP		1/10 - 1/100.

a	u	L

Function

Core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I) that is believed to belong to the minimal assembly required for 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.

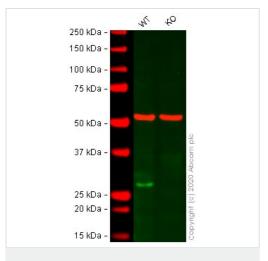
Sequence similarities

Belongs to the complex I 30 kDa subunit family.

Cellular localization

Mitochondrion inner membrane.

Images



Western blot - Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471)

All lanes : Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: NDUFS3 knockout HEK-293T cell lysate

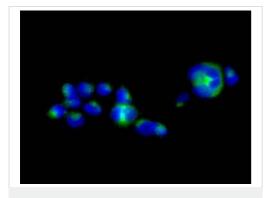
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 30 kDa **Observed band size:** 27 kDa

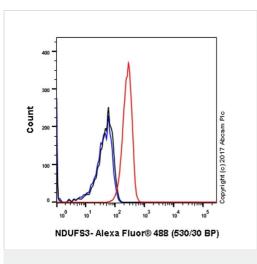
Lanes 1 - 2: Merged signal (red and green). Green - ab177471 observed at 27 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab177471 was shown to react with NDUFS3 in HEK-293T wild-type cells in western blot with loss of signal observed in NDUFS3 knockout cell line ab266419 (NDUFS3 knockout cell lysate ab257556). Wild-type and NDUFS3 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab177471 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



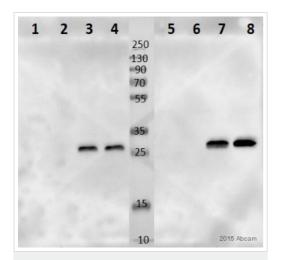
Immunocytochemistry/ Immunofluorescence - Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471)

Immunofluorescence analysis of 293 cells labeling NDUFS3 with ab177471 at 1/50 dilution (green). DAPI nuclear staining (blue).



Flow Cytometry (Intracellular) - Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471)

Intracellular Flow Cytometry analysis of HEK293 (Human embryonic kidney epithelial cell) cells labeling NDUFS3 (red) with purified ab177471 at a 1/200 dilution (10ug/mL). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti rabbit lgG (Alexa Fluor[®]488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal lgG (Black) (**ab172730**). Blue (unlabeled control) - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471)

Image is courtesy of an anonymous Abreview.

All lanes : Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471) at 1/1000 dilution

Lanes 1-2: Whole cell lysates from NDUFS3 KO cells.Lanes 3-4: Whole cell lysates from NDUFS3 WT cells.Lanes 5-6: Isolated mitochondria from NDUFS3 KO Cells.

Lanes 7-8: Isolated mitochondria from NDUFS3 WT Cells.

Lysates/proteins at 50 µg per lane.

Secondary

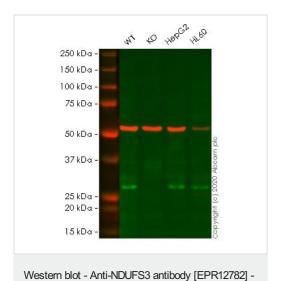
All lanes : Goat anti-Rabbit polyclonal HRP conjugate. at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 30 kDa

Exposure time: 1 minute



C-terminal (ab177471)

All lanes : Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: NDUFS3 knockout HEK-293T cell lysate

Lane 3 : HepG2 cell lysate

Lane 4 : HL60 cell lysate

Lysates/proteins at 20 µg per lane.

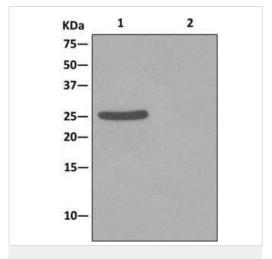
Performed under reducing conditions.

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

Loading Control (ab7291) observed at 50 kDa.

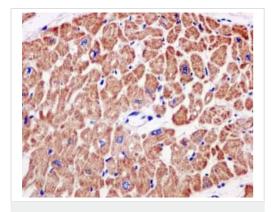
ab177471 was shown to react with NDUFS3 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab266419 (knockout cell lysate ab257556) was used. Wild-type HEK-293T and NDUFS3 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab177471 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

Lanes 1-4: Merged signal (red and green). Green - ab177471 observed at 30 kDa. Red - Anti-alpha Tubulin antibody [DM1A] -



Immunoprecipitation - Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471)

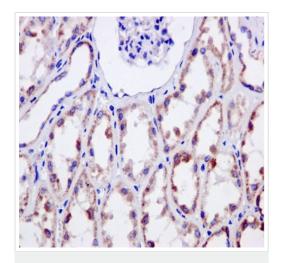
Western blot analysis on immunoprecipitation pellet from (1) human fetal heart lysate or (2) 1XPBS (negative control) using ab177471at 1/10 dilution, and HRP-conjugated anti-rabbit lgG preferentially detecting the non-reduced form of rabbit lgG.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NDUFS3 antibody
[EPR12782] - C-terminal (ab177471)

Immunohistochemcal analysis of paraffin-embedded Human heart tissue labeling NDUFS3 with ab177471 at 1/50 dilution.

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

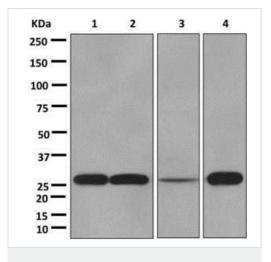


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NDUFS3 antibody

[EPR12782] - C-terminal (ab177471)

Immunohistochemcal analysis of paraffin-embedded Human kidney tissue labeling NDUFS3 with ab177471 at 1/50 dilution.

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



Western blot - Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471)

All lanes : Anti-NDUFS3 antibody [EPR12782] - C-terminal (ab177471) at 1/1000 dilution

Lane 1 : HepG2 lysate
Lane 2 : 293T lysate
Lane 3 : HL60 lysate
Lane 4 : Fetal heart lysate

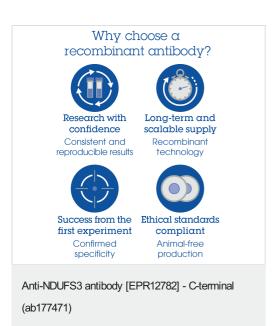
Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat-anti-rabbit HRP at 1/2000 dilution

Developed using the ECL technique.

Predicted band size: 30 kDa



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