abcam

Product datasheet

Anti-NCE2/UBE2F antibody [EPR12932] ab185234





4 References 7 Images

Overview

Product name Anti-NCE2/UBE2F antibody [EPR12932]

Rabbit monoclonal [EPR12932] to NCE2/UBE2F **Description**

Host species Rabbit

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

Species reactivity Reacts with: Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, Jurkat, A375 and NTERA2/D1 cell lysates. Flow Cyt (intra): A375 cells. ICC/IF: A375

cells. IHC-P: Human brain and Human renal carcinoma tissues.

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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR12932

Isotype ΙgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab185234 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)		1/160. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/1000. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/250.

Function Accepts the ubiquitin-like protein NEDD8 from the UBA3-NAE1 E1 complex and catalyzes its

covalent attachment to other proteins. The specific interaction with the E3 ubiquitin ligase RBX2,

but not RBX1, suggests that the RBX2-UBE2F complex neddylates specific target proteins, such

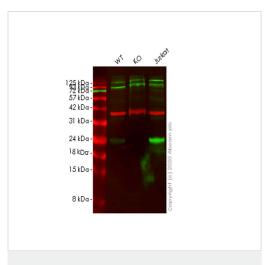
as CUL5.

Tissue specificity Widely expressed (at protein level).

 Pathway
 Protein modification; protein neddylation.

Sequence similaritiesBelongs to the ubiquitin-conjugating enzyme family. UBE2F subfamily.

Images



Western blot - Anti-NCE2/UBE2F antibody [EPR12932] (ab185234)

All lanes : Anti-NCE2/UBE2F antibody [EPR12932] (ab185234) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: UBE2F knockout HeLa cell lysate

Lane 3: Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

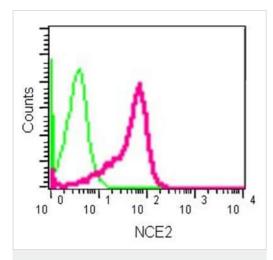
Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 21 kDa
Observed band size: 24 kDa

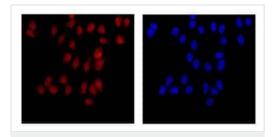
Lanes 1-3: Merged signal (red and green). Green - ab185234 observed at 24 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab185234 Anti-NCE2/UBE2F antibody [EPR12932] was shown to specifically react with NCE2/UBE2F in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265339 (knockout cell line ab265339 (knockout cell lysate ab265339 (knockout cell lysate ab265339 (knockout cell line ab2657777) was used. Wild-type and NCE2/UBE2F knockout samples were subjected to SDS-PAGE. ab185234 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



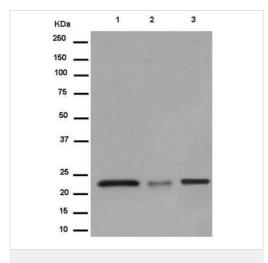
Flow Cytometry (Intracellular) - Anti-NCE2/UBE2F antibody [EPR12932] (ab185234)

Intracellular flow cytometric analysis of A375 cells (2% paraformaldehyde-fixed) labeling NCE2/UBE2F with ab185234at 1/160 dilution (red) or a rabbit lgG (negative) (green), followed by Goat anti rabbit lgG (FITC) secondary at 1/150 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-NCE2/UBE2F antibody [EPR12932] (ab185234)

Immunofluorescent analysis of A375 cells (4% paraformaldehydefixed) labeling NCE2/UBE2F with ab185234 at 1/250 dilution followed by Goat anti rabbit lgG (AlexaFluor® 555) secondary at 1/200 dilution and counter-stained with DAPI (blue).



Western blot - Anti-NCE2/UBE2F antibody [EPR12932] (ab185234)

All lanes : Anti-NCE2/UBE2F antibody [EPR12932] (ab185234) at 1/1000 dilution

Lane 1 : Jurkat cell lysate

Lane 2 : A375 cell lysate

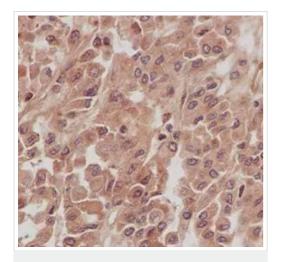
Lane 3: NTERA2/D1 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

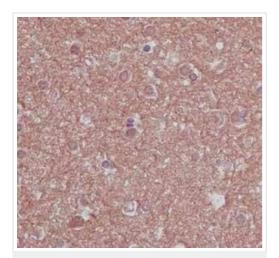
Predicted band size: 21 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NCE2/UBE2F antibody [EPR12932] (ab185234)

Immunohistochemical analysis of paraffin-embedded Human renal carcinoma labeling NCE2/UBE2F with ab185234 at 1/100 dilution followed by pre-diluted HRP-conjugated secondary antibody and counter-stained with Hematoxylin.

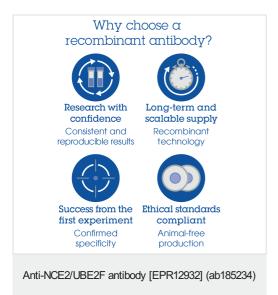
Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NCE2/UBE2F antibody [EPR12932] (ab185234)

Immunohistochemical analysis of paraffin-embedded Human brain labeling NCE2/UBE2F with ab185234 at 1/100 dilution followed by pre-diluted HRP-conjugated secondary antibody and counterstained with Hematoxylin.

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



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