abcam

Product datasheet

Anti-KPNB1 antibody [3E9] ab2811

19 Abreviews 35 References 11 Images

Overview

Product name	Anti-KPNB1 antibody [3E9]
Description	Mouse monoclonal [3E9] to KPNB1
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, ChIP, IP, Inhibition Assay, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Cow, Dog, Human, Pig, African green monkey, Syrian hamster
Immunogen	Full length native protein (purified) corresponding to Cow KPNB1. Purified from Bovine erythrocytes.
Positive control	WB: MDBK cell lysate ICC: MDBK cells, PTK cells
General notes	Previously labelled as NTF97/Importin beta.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituent: PBS
Purity	Protein A purified
Primary antibody notes	The accumulation of proteins in the nucleus is mediated by short sequences of basic amino acids called nuclear localization sequences (NLSs). These sequences are necessary and sufficient to direct a protein or inert carrier to the nuclear interior. Nuclear protein import is accomplished by two sequential, energy dependent events. The first step, docking at the nuclear pore complex, requires a 54/56 kDa nuclear localization signal receptor (a-karyopherin, importin-a, SRP-a) and the nuclear transport factor p97 (NTF 97, b-karyopherin, importin-b). The second step, translocation across the nuclear envelope (NE), requires the GTP-binding protein, Ran/TC4.
Clonality	Monoclonal
Clone number	3E9
Isotype	IgG2a

Applications

Our Abpromise guarantee covers the use of **ab2811** 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		1/100. ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
ChIP		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Inhibition Assay		Use at an assay dependent concentration.
WB	★★★★★	1/5000. Detects a band of approximately 97 kDa (predicted molecular weight: 97 kDa).
ICC/IF	★★★★★	1/1000.

Target

Function

Functions in nuclear protein import, either in association with an adapter protein, like an importin-alpha subunit, which binds to nuclear localization signals (NLS) in cargo substrates, or by acting as autonomous nuclear transport receptor. Acting autonomously, serves itself as NLS receptor. Docking of the importin/substrate complex to the nuclear pore complex (NPC) is mediated by KPNB1 through binding to nucleoporin FxFG repeats and the complex is subsequently translocated through the pore by an energy requiring, Ran-dependent mechanism. At the nucleoplasmic side of the NPC, Ran binds to importin-beta and the three components separate and importin-alpha and -beta are re-exported from the nucleus to the cytoplasm where GTP hydrolysis releases Ran from importin. The directionality of nuclear import is thought to be conferred by an asymmetric distribution of the GTP- and GDP-bound forms of Ran between the cytoplasm and nucleus. Mediates autonomously the nuclear import of ribosomal proteins RPL23A, RPS7 and RPL5. Binds to a beta-like import receptor binding (BIB) domain of RPL23A. In association with IPO7 mediates the nuclear import of H1 histone. In vitro, mediates nuclear import of H2A, H2B, H3 and H4 histones. In case of HIV-1 infection, binds and mediates the nuclear import of HIV-1 Rev. Imports PRKCI into the nucleus.

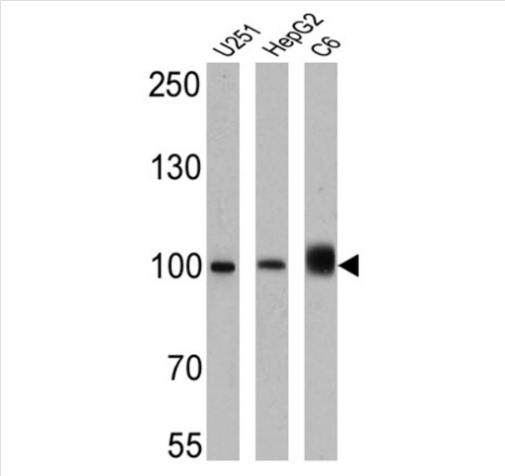
Sequence similarities

Belongs to the importin beta family.
Contains 8 HEAT repeats.
Contains 1 importin N-terminal domain.

Cellular localization

Cytoplasm. Nucleus envelope.

Images



Western blot - Anti-KPNB1 antibody [3E9] (ab2811)

All lanes : Anti-KPNB1 antibody [3E9] (ab2811)

Lane 1 : U251 cell lysate

Lane 2 : HepG2 cell lysate

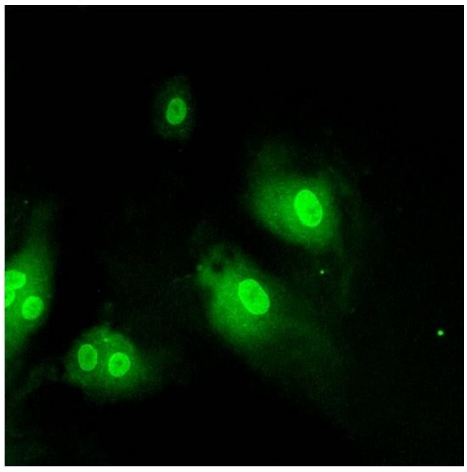
Lane 3 : C6 cell lysate

Lysates/proteins at 25 µg per lane.

Predicted band size: 97 kDa

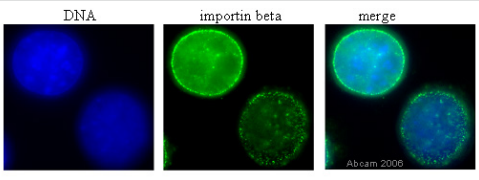
Observed band size: 100 kDa

why is the actual band size different from the predicted?



Immunocytochemistry/ Immunofluorescence - Anti-KPNB1 antibody [3E9] (ab2811)

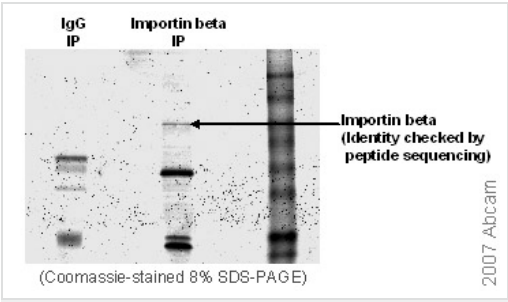
Immunofluorecent analysis of HMVEC (Human Lung Microvascular Endothelial cells) cells stained for KPNB1 using ab2811.



Immunocytochemistry/ Immunofluorescence - Anti-KPNB1 antibody [3E9] (ab2811)

This image is courtesy of Roberto Giambruno, Marilena Ciciarello and Patrizia Lavia

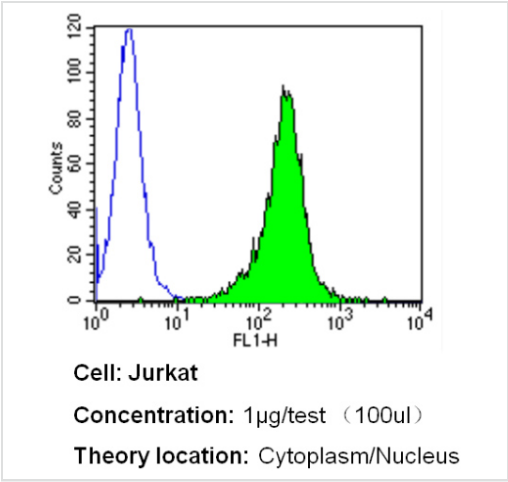
NIH3T3 cells were incubated for 4 minutes in PHEM/1%triton, washed for 2 minutes in 1x PHEM and fixed for 10 minutes at room temperature in 3.7% PFA containing 30mM sucrose. Following washing in PBS, the cells were incubated for 2 minutes in 100% Methanol at -20°C, then washed 3 times in PBS. The cells were then incubated with ab2811 (1/200) for 1 hour at room temperature. The image panel shows the nuclei stained with DAPI (blue) and the nuclear envalope and cytoplasm stained with ab2811 (green). 100x magnification.



Immunoprecipitation - Anti-KPNB1 antibody [3E9] (ab2811)

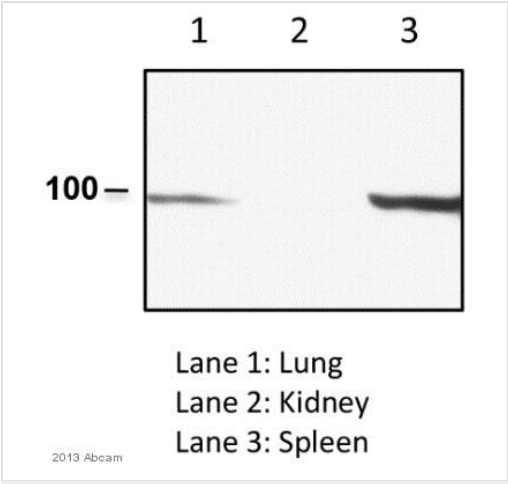
Image and protocol courtesy of Rosamaria Mangiacasale and Patrizia Lavia, University 'La Sapienza' CNR, Italy

Immunoprecipitation of Importin beta, in HeLa cells, using ab2811. Coomassie-stained 8% SDS-page gel was loaded with IP fractions obtained by incubating 2 mg of pre-cleared HeLa whole cell extracts with 4µg ab2811 or 4µg IgG (control). The Importin band (see arrow) was cut out of the gel and its identity confirmed by Mass Spectometry. Please refer to protocol tab for further experimental details.



Flow Cytometry - Anti-KPNB1 antibody [3E9] (ab2811)

Flow cytometry analysis of KPNB1 in Jurkat cells (green) compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of 1-5x10^6 cells/ml, fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2811 (1ug/test) for 40 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated secondary antibody and re-suspended in PBS for FACS analysis.



Western blot - Anti-KPNB1 antibody [3E9] (ab2811)

This image is courtesy of an anonymous Abreview

All lanes : Anti-KPNB1 antibody [3E9] (ab2811) at 1/2000 dilution

Lane 1 : Mouse lung whole tissue lysate
Lane 2 : Mouse kidney whole tissue lysate
Lane 3 : Mouse spleen whole tissue lysate

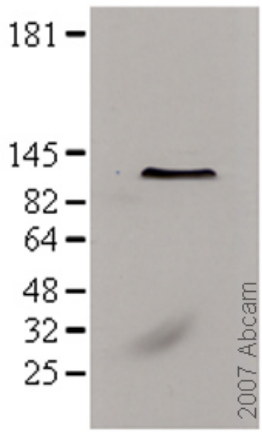
Secondary
All lanes : HRP-conjugated Goat anti-mouse IgG polyclonal at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 97 kDa
Observed band size: 97 kDa

Exposure time: 3 minutes



Western blot - Anti-KPNB1 antibody [3E9] (ab2811)

Image and protocol courtesy of Rosamaria Mangiacasale and Patrizia Lavia, University 'La Sapienza' CNR, Italy

Anti-KPNB1 antibody [3E9] (ab2811) at 1/5000 dilution + HeLa Whole cell extract

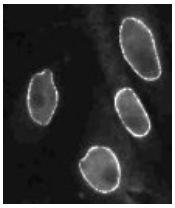
Secondary

HRP-conjugated anti mouse IgG at 1/5000 dilution

Performed under reducing conditions.

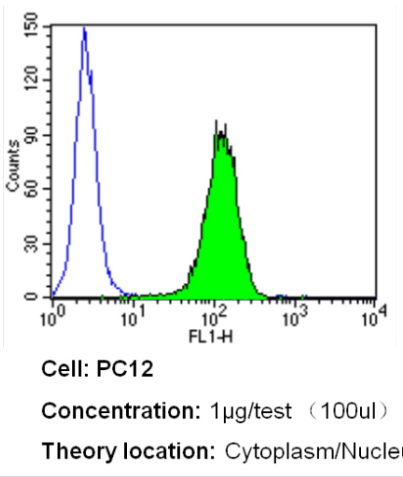
Predicted band size: 97 kDa

HeLa whole cell extract was run on a 10%SDS-PAGE and transferred to PVDF membrane. Membrane was blocked for 30 mins in TBS/0.1% Tween/ 5% Milk; ab2811 (1/5000) was incubated for 1 hr in TBS/0.1%Tween/5% Milk and followed by 3 washes in TBS/ 0.1%Tween (3x 7 mins). Secondary antibody was incubated for 30 mins in a TBS/ 0.1% Tween solution.This was followed by 3 washes with the TBST solution (3x7 mins) and one wash in TBS. Western blot developed using ECL plus (Amersham).



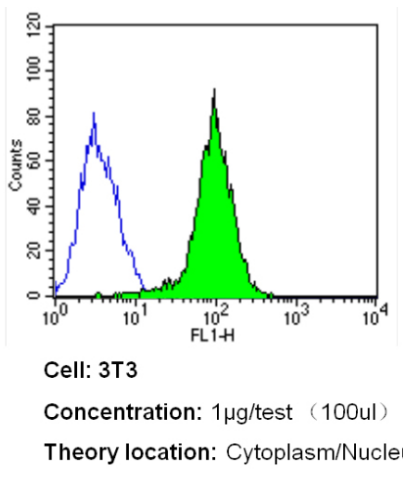
Immunocytochemistry/ Immunofluorescence - Anti-KPNB1 antibody [3E9] (ab2811)

Immunolocalization of KPNB1 in PTK cells using ab2811.



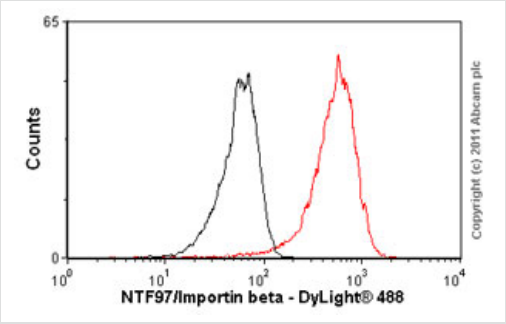
Flow Cytometry - Anti-KPNB1 antibody [3E9] (ab2811)

Flow cytometry analysis of KPNB1 in PC12 cells (green) compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of 1-5x10^6 cells/ml, fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2811 (1ug/test) for 40 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated secondary antibody and re-suspended in PBS for FACS analysis.



Flow Cytometry - Anti-KPNB1 antibody [3E9] (ab2811)

Flow cytometry analysis of KPNB1 in 3T3 cells (green) compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of 1-5x10^6 cells/ml, fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2811 (1ug/test) for 40 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated secondary antibody and re-suspended in PBS for FACS analysis.



Flow Cytometry - Anti-KPNB1 antibody [3E9] (ab2811)

Overlay histogram showing Jurkat cells stained with ab2811 (red line). The cells were fixed with 4% paraformaldehyde (10 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 (ab2811, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors