

Anti-Exportin-5 antibody [2C5-1B3] α b57491

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Overview

Product name	Anti-Exportin-5 antibody [2C5-1B3]
Description	Mouse monoclonal [2C5-1B3] to Exportin-5
Host species	Mouse
Tested applications	Suitable for: WB, Flow Cyt, IP, ICC/IF, IHC-P
Species reactivity	Reacts with: Human
Immunogen	Recombinant full length protein, corresponding to amino acids 1-281 of Human Exportin-5
General notes	<p>This product was changed from ascites to tissue culture supernatant on 15 May 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.</p> <p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.4
Purity	Tissue culture supernatant
Purification notes	Purified from TCS.
Clonality	Monoclonal
Clone number	2C5-1B3
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab57491 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
WB		Use at an assay dependent concentration. Predicted molecular weight: 136 kDa.
Flow Cyt		Use at an assay dependent concentration. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Target

Function

Mediates the nuclear export of proteins bearing a double-stranded RNA binding domain (dsRBD) and double-stranded RNAs (cargos). XPO5 in the nucleus binds cooperatively to the RNA and to the GTPase Ran in its active GTP-bound form. Proteins containing dsRBDs can associate with this trimeric complex through the RNA. Docking of this complex to the nuclear pore complex (NPC) is mediated through binding to nucleoporins. Upon transit of a nuclear export complex into the cytoplasm, hydrolysis of Ran-GTP to Ran-GDP (induced by RANBP1 and RANGAP1, respectively) cause disassembly of the complex and release of the cargo from the export receptor. XPO5 then returns to the nuclear compartment by diffusion through the nuclear pore complex, to mediate another round of transport. The directionality of nuclear export is thought to be conferred by an asymmetric distribution of the GTP- and GDP-bound forms of Ran between the cytoplasm and nucleus. Overexpression may in some circumstances enhance RNA-mediated gene silencing (RNAi).

Mediates the nuclear export of micro-RNA precursors, which form short hairpins. Also mediates the nuclear export of synthetic short hairpin RNAs used for RNA interference, and adenovirus VA1 dsRNA. In some circumstances can also mediate the nuclear export of deacylated and aminoacylated tRNAs. Specifically recognizes dsRNAs that lack a 5'-overhang in a sequence-independent manner, have only a short 3'-overhang, and that have a double-stranded length of at least 15 base-pairs. Binding is dependent on Ran-GTP.

Tissue specificity

Expressed in heart, brain, placenta, lung, skeletal muscle, kidney and pancreas.

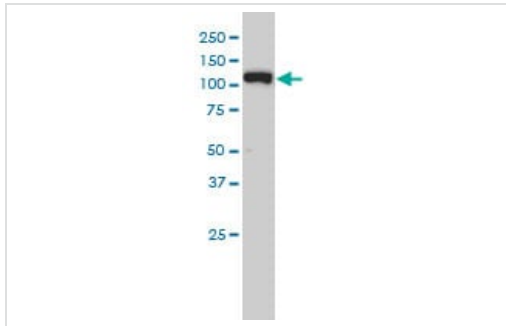
Sequence similarities

Belongs to the exportin family.

Cellular localization

Nucleus. Cytoplasm. Shuttles between the nucleus and the cytoplasm.

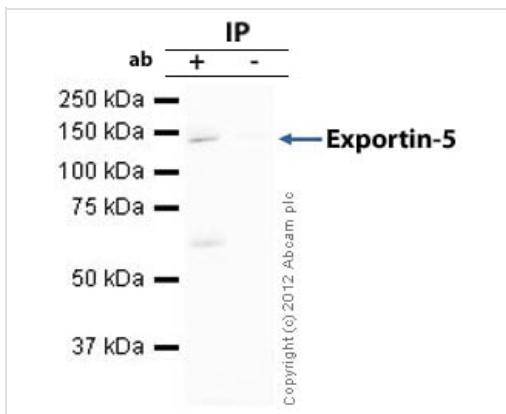
Images



Western blot - Anti-Exportin-5 antibody [2C5-1B3] (ab57491)

Exportin-5 antibody (ab57491) at 1ug/lane + HeLa cell lysate at 25ug/lane.

This image was generated using the ascites version of the product.



Immunoprecipitation - Anti-Exportin-5 antibody [2C5-1B3] (ab57491)

Exportin-5 was immunoprecipitated using 0.5mg Hek293 whole cell extract, 10µg of Mouse monoclonal to Exportin-5 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

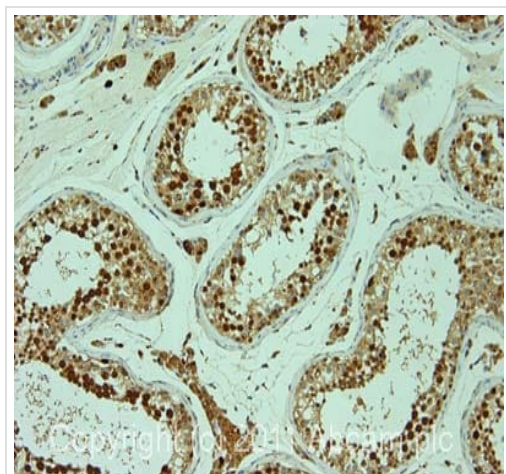
The antibody was incubated under agitation with Protein G beads for 10min, Hek293 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab57491.

Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/5000 dilution.

Band: 140kDa: Exportin-5; non specific - 65kDa: We are unsure as to the identity of this extra band.

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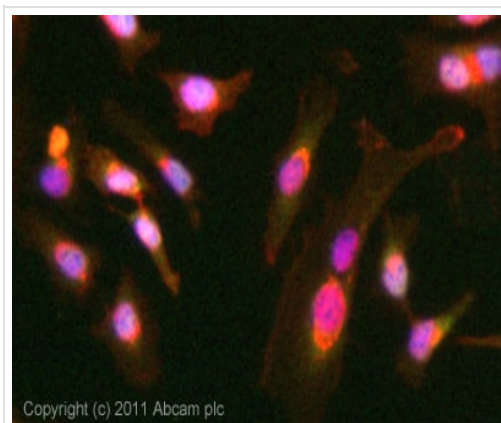


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Exportin-5 antibody [2C5-1B3] (ab57491)

IHC image of ab57491 staining in normal human testis formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab57491, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

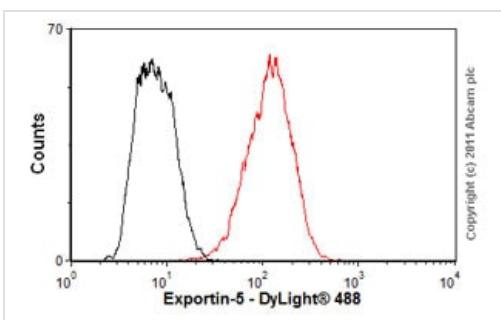
This image was generated using the ascites version of the product.



Immunocytochemistry/ Immunofluorescence - Anti-Exportin-5 antibody [2C5-1B3] (ab57491)

ICC/IF image of ab57491 stained HeLa cells. The cells were 4% formaldehyde (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab57491, 10µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96879** Dylight 488 goat anti-mouse IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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Flow Cytometry - Anti-Exportin-5 antibody [2C5-1B3] (ab57491)

Overlay histogram showing HEK293 cells stained with ab57491 (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 (ab57491, 1µg/1x10⁶ cells) 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 IgG1 [ICIGG1] (**ab91353**, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.

This image was generated using the ascites version of the product.

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