# abcam

# Product datasheet

# Anti-Importin subunit alpha antibody [EPR26346-53] ab307438



重组 RabMAb

# 13 图像

### 概述

产品名称 Anti-Importin subunit alpha抗体[EPR26346-53]

描述 兔单克隆抗体[EPR26346-53] to SRP1

宿主 Rabbit

Unsuitable for mouse and rat IHC-P. 特异性

适用于: Flow Cyt (Intra), WB, ICC/IF, IHC-P, IP 经测试应用

种属反应性 与反应: Mouse, Rat, Human

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

阳性对照 WB: Mouse and rat brain tissue lysate. NIH/3T3, HepG2, Jurkat and HeLa whole cell lysate.

> Human cerebellum, heart and kidney tissue lysate. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (Intra): HepG2 and NIH/3T3 cells. IHC-P: Human breast and breast cancer tissue. Human

colorectal adenocarcinoma. IP: HepG2 whole cell lysate.

常规说明 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**.

### 性能

形式 Liquid

存放说明 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.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

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

纯度 Protein A purified

克隆 单克隆

**克隆编号** EPR26346-53

**同种型** IgG

### 应用

### The Abpromise guarantee Abpromise™承诺保证使用ab307438于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/500.
WB		1/1000. Predicted molecular weight: 60 kDa.
ICC/IF		1/50.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

### 靶标

### 功能

Functions in nuclear protein import as an adapter protein for nuclear receptor KPNB1. Binds specifically and directly to substrates containing either a simple or bipartite NLS motif. 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. In vitro, mediates the nuclear import of human cytomegalovirus UL84 by recognizing a non-classical NLS.

组织特异性

Expressed ubiquitously.

序列相似性

Belongs to the importin alpha family.

Contains 10 ARM repeats.

Contains 1 IBB domain.

结构域

Consists of an N-terminal hydrophilic region, a hydrophobic central region composed of 10 repeats, and a short hydrophilic C-terminus. The N-terminal hydrophilic region contains the importin beta binding domain (IBB domain), which is sufficient for binding importin beta and essential for nuclear protein import.

The IBB domain is thought to act as an intrasteric autoregulatory sequence by interacting with the internal autoinhibitory NLS. Binding of KPNB1 probably overlaps the internal NLS and contributes to a high affinity for cytoplasmic NLS-containing cargo substrates. After dissociation of the importin/substrate complex in the nucleus the internal autohibitory NLS contributes to a low affinity for nuclear NLS-containing proteins.

The major and minor NLS binding sites are mainly involved in recognition of simple or bipartite NLS motifs. Structurally located within in a helical surface groove they contain several conserved

Trp and Asn residues of the corresponding third helices (H3) of ARM repeats which mainly contribute to binding.

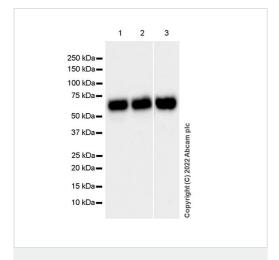
### 翻译后修饰

Polyubiquitinated in the presence of RAG1 (in vitro).

细胞定位

Cytoplasm. Nucleus.

### 图片



Western blot - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

**All lanes :** Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate

Lane 2: Rat brain tissue lysate

Lane 3: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

### **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 60 kDa Observed band size: 60 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 37 seconds.

**All lanes :** Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438) at 1/1000 dilution

**Lane 1 :** HeLa (human cervix adenocarcinoma epithelial cell) transfected with scrambled siRNA control whole cell lysate

Lane 2: HeLa transfected with siRNA specifically targeting SRP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

# 1 2 250 kDa — 150 kDa — 100 kDa — 75 kDa — 50 kDa — 37 kDa — 25 kDa — 39d 20 kDa — 29d 20 kDa — 29d 20 kDa — 29d 20 kDa — 20d 10 kDa

Western blot - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

# Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 60 kDa

Observed band size: 60 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, anti-GAPDH antibody (<u>ab181602</u>) loading control staining at 1/200000 dilution.

Exposure time: 39 seconds.

**All lanes :** Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438) at 1/1000 dilution

Lane 1: Human cerebellum tissue lysate

Lane 2 : Human heart tissue lysate

Lane 3 : Human kidney tissue lysate

Lysates/proteins at 20 µg per lane.

1 2 3

250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

Western blot - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

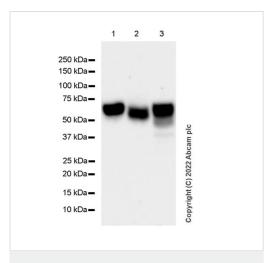
# Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

**Predicted band size:** 60 kDa **Observed band size:** 60 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 147 seconds.



Western blot - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

**All lanes :** Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438) at 1/1000 dilution

**Lane 1 :** HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate

**Lane 2**: HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate

**Lane 3**: Jurkat (human T cell leukemia T lymphocyte from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

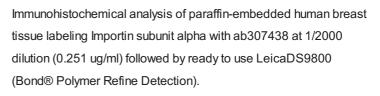
### **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 60 kDa
Observed band size: 60 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 26 seconds.

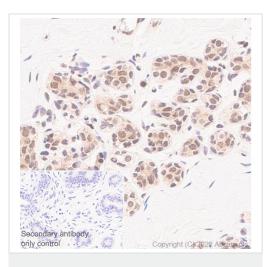


Positive staining on human breast (PMID: 34709479).

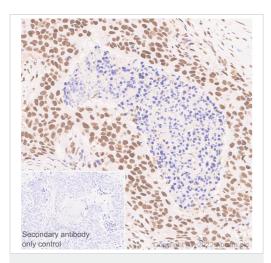
The section was incubated with ab307438 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling Importin subunit alpha with ab307438 at 1/2000 dilution (0.251 ug/ml) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

Positive staining on human breast cancer (PMID: 34709479).

The section was incubated with ab307438 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

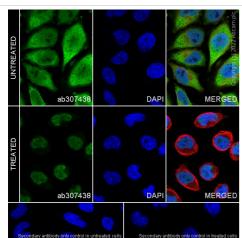
Immunohistochemical analysis of paraffin-embedded human colorectal adenocarcinoma tissue labeling Importin subunit alpha with ab307438 at 1/2000 dilution (0.251 ug/ml) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

Positive staining on human colorectal adenocarcinoma.

The section was incubated with ab307438 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

UNTREATED

REATED

ab307438

ab307438

ug/ml) (Green).

Immunocytochemistry/ Immunofluorescence - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

DAPI

MERGED

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervical adenocarcinoma epithelial cell) cells labelling Importin subunit alpha with ab307438 at 1/50 dilution (10.02 ug/ml) followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 ug/ml) (Green).

Confocal image showing cytoplasmic and nuclear staining in untreated HeLa cells, and the signals were translocated to nucleus in HeLa cells after treatment with heat shock (45.5°C) for 1.5 h.

Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 ug/ml) (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (2 ug/ml).

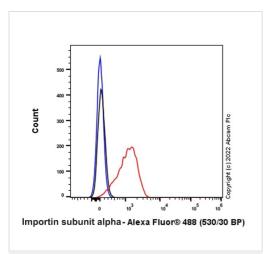
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling Importin subunit alpha with ab307438 at 1/50 dilution (10.02 ug/ml) followed by ab150081 Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2

Confocal image showing cytoplasmic and nuclear staining in untreated NIH/3T3 cells, and the signals were translocated to nucleus in NIH/3T3 cells after treatment with heat shock (45.5°C) for

Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 ug/ml) (Red). The nuclear counterstain was DAPI (Blue).

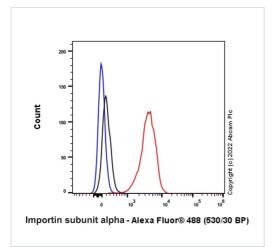
Secondary antibody only control: Secondary antibody is ab150081

Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (2 ug/ml).



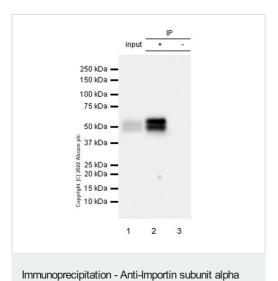
Flow Cytometry (Intracellular) - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HepG2 (human hepatocellular carcinoma epithelial cell) cells labelling Importin subunit alpha with ab307438 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Importin subunit alpha antibody [EPR26346-53] (ab307438)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling Importin subunit alpha with ab307438 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



antibody [EPR26346-53] (ab307438)

Importin subunit alpha was immunoprecipitated from 0.35 mg HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate with ab307438 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab307438 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

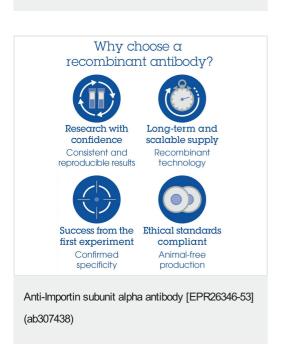
Lane 1: HepG2 whole cell lysate 10ug

Lane 2: ab307438 IP in HepG2 whole cell lysate

**Lane 3:** Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab307438 in HepG2 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.



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