abcam

ab240326

Anti-Lin28B antibody [EPR18717] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR18717
Purification technique	Affinity purification Protein A
Concentration	1.026 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

WB

Tested

Species	Human, Recombinant full length protein - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

Target data

See full target information LIN28B

Function	Suppressor of microRNA (miRNA) biogenesis, including that of let-7 and possibly of miR107, miR-143 and miR-200c. Binds primary let-7 transcripts (pri-let-7), including pri-let-7g and pri-let-7a-1, and sequester them in the nucleolus, away from the microprocessor complex, hence preventing their processing into mature miRNA (PubMed:22118463). Does not act on pri-miR21 (PubMed:22118463). The repression of let-7 expression is required for normal development and contributes to maintain the pluripotent state of embryonic stem cells by preventing let-7-mediated differentiation. When overexpressed, recruits ZCCHC11/TUT4 uridylyltransferase to pre-let-7 transcripts, leading to their terminal uridylation and degradation (PubMed:19703396). This activity might not be relevant in vivo, as LIN28B-mediated inhibition of let-7 miRNA maturation appears to be ZCCHC11-independent (PubMed:22118463). Interaction with target pre-miRNAs occurs via an 5'-GGAG-3' motif in the pre-miRNA terminal loop. Mediates MYC-induced let-7 repression (By similarity). When overexpressed, isoform 1 stimulates growth of the breast adenocarcinoma cell line MCF-7. Isoform 2 has no effect on cell growth.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab240326 is the carrier-free version of ab191881.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

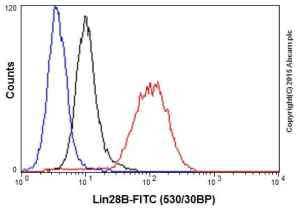
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

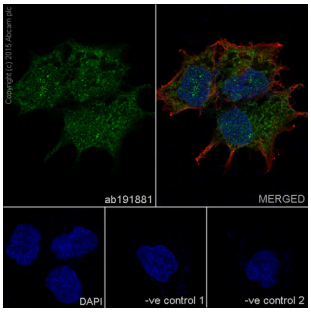
7 product images



Flow Cytometry (Intracellular) - Anti-Lin28B antibody [EPR18717] - BSA and Azide free (ab240326)

Lin28B Flow Cytometry (Intracellular) staining using rabbit Anti-Lin28B antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling Lin28B with ab191881 at 1/150 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191881).

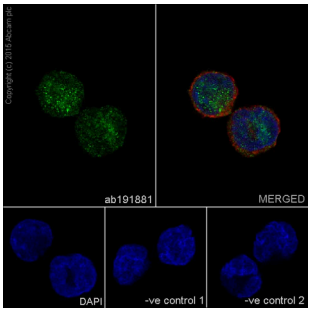


Immunocytochemistry/ Immunofluorescence - Anti-Lin28B antibody [EPR18717] - BSA and Azide free (ab240326)

Lin28B Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Lin28B antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized JAR (Human placenta choriocarcinoma cell line) cells labeling Lin28B with ab191881 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasm and nuclear staining on JAR cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).
The negative controls are as follows:
-ve control 1: ab191881 at 1/1000 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.
-ve control 2: ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191881).



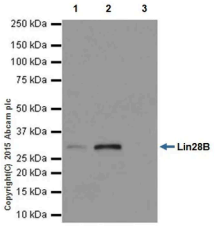
Immunocytochemistry/ Immunofluorescence - Anti-Lin28B antibody [EPR18717] - BSA and Azide free (ab240326)

Lin28B Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Lin28B antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling Lin28B with ab191881 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasm and nuclear staining on K562 cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).
The negative controls are as follows:
-ve control 1: ab191881 at 1/1000 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution.

-ve control 2: ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191881).



Immunoprecipitation - Anti-Lin28B antibody [EPR18717] - BSA and Azide free (ab240326)

Lin28B was immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate with ab191881 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab191881 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.
Lane 1: K562 whole cell lysate 10ug (Input).

Lane 2: ab191881 IP in K562 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab191881 in K562 whole cell lysate.

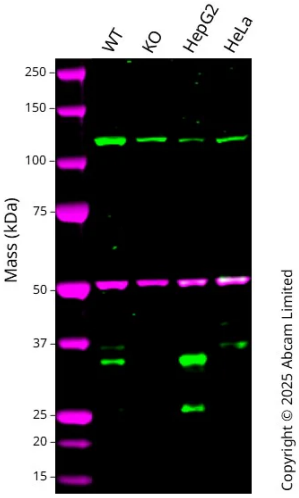
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 5 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191881).

All lanes:
Immunoprecipitation - Anti-Lin28B antibody [EPR18717] (ab191881)

Predicted band size: 27 kDa



Western blot - Anti-Lin28B antibody [EPR18717] - BSA and Azide free (ab240326)

Lin28B Western blot staining using rabbit Anti-Lin28B antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191881).

Western blot: Anti-Lin28B antibody [EPR18717] ab191881 staining at 1/2000 dilution, shown in green; Mouse anti alpha Tubulin ab7291 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 34 kDa in Wild-type A549 cell lysates with no signal observed at this size in LIN28B knockout A549 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-Lin28B antibody [EPR18717] (ab191881) at 1/2000 dilution

Lane 1:
Wild-type A549 whole cell lysate at 20 µg

Lane 2:
Western blot - Human LIN28B knockout A549 cell line (ab288867) at 20 µg

Lane 3:

HepG2 whole cell lysate at 20 µg

Lane 4:
HeLa whole cell lysate at 20 µg

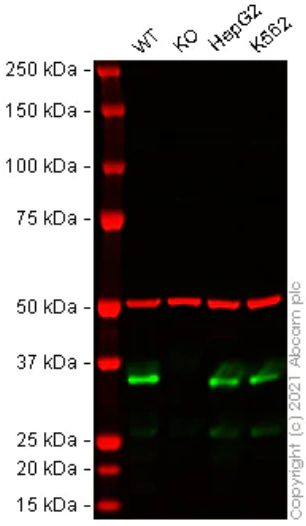
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 27 kDa

Observed band size: 34 kDa



Western blot - Anti-Lin28B antibody [EPR18717] - BSA and Azide free (ab240326)

This data was developed using the same antibody clone in a different buffer formulation (ab191881).
Lanes 1 - 4: Merged signal (red and green). Green - ab191881 observed at 35 kDa. Red - loading control ab7291 (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab191881 was shown to react with Lin28B in wild-type HEK-293 cells in Western blot with loss of signal observed in LIN28B knockout sample. Wild-type HEK-293 and LIN28B knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab191881 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 2000 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 h at room temperature before imaging.

All lanes:
Western blot - Anti-Lin28B antibody [EPR18717] (ab191881) at 1/2000 dilution

Lane 1:
Wild-type HEK293 cell lysate at 20 µg

Lane 2:
LIN28B knockout HEK293 cell lysate at 20 µg

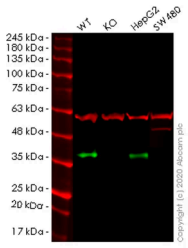
Lane 3:
HepG2 cell lysate at 20 µg

Lane 4:
K562 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 27 kDa

Observed band size: 35 kDa



Western blot - Anti-Lin28B antibody [EPR18717] - BSA and Azide free (ab240326)

Lin28B Western blot staining using rabbit Anti-Lin28B antibody

This data was developed using the same antibody clone in a different buffer formulation (ab191881).
Lanes 1-4: Merged signal (red and green). Green - ab191881 observed at 36 kDa. Red - loading control ab7291 observed at 50 kDa.

ab191881 Anti-Lin28B antibody [EPR18717] was shown to specifically react with Lin28B in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab265066 (knockout cell lysate ab257504) was used. Wild-type and Lin28B knockout samples were subjected to SDS-PAGE. ab191881 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) were incubated overnight at 4°C at 1 in 1000 dilution and 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.

All lanes:
Western blot - Anti-Lin28B antibody [EPR18717] (ab191881) at 1/1000 dilution

Lane 1:
Wild-type HEK293T cell lysate at 20 µg

Lane 2:
LIN28B knockout HEK293T cell lysate at 20 µg

Lane 3:
HepG2 cell lysate at 20 µg

Lane 4:
SW480 cell lysate at 20 µg

Secondary

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

Predicted band size: 27 kDa

Observed band size: 36 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.