abcam

ab191881

Anti-Lin28B antibody [EPR18717]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR18717
Purification technique	Affinity purification Protein A
Concentration	0.584 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/50
Notes	-

Not recommended

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

WB

Tested

Species	Recombinant full length protein - Human
Dilution info	1/2000
Notes	-

Species	Human
Dilution info	1/2000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1-10 µg/mL
Notes	-

Not recommended

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/150

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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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.

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

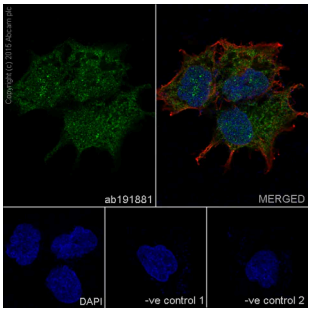
We do not recommend this combination. It is not covered by our product promise.

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.

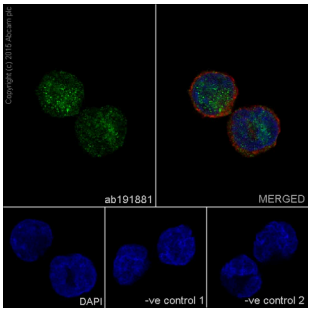
13 product images



Immunocytochemistry/ Immunofluorescence - Anti-Lin28B antibody [EPR18717] (ab191881)

Lin28B Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Lin28B antibody

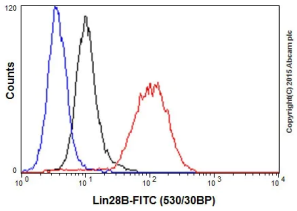
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized JAR (Human placenta choriocarcinoma cell line) cells labeling Lin28B with ab191881 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). 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.



Immunocytochemistry/ Immunofluorescence - Anti-Lin28B antibody [EPR18717] (ab191881)

Lin28B Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Lin28B antibody

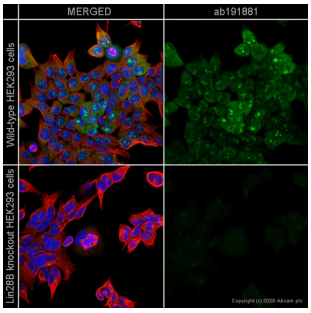
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling Lin28B with ab191881 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 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.



Flow Cytometry (Intracellular) - Anti-Lin28B antibody [EPR18717] (ab191881)

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

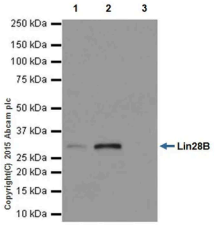
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling Lin28B with ab191881 at 1/150 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Lin28B antibody [EPR18717] (ab191881)

Lin28B Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Lin28B antibody

ab191881 staining Lin28B in wild-type HEK293 cells (top panel) and Lin28B knockout HEK293 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab191881 at 10µg/ml and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a high-content analysis system (Perkin Elmer, Operetta CLS™).



Immunoprecipitation - Anti-Lin28B antibody [EPR18717] (ab191881)

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.

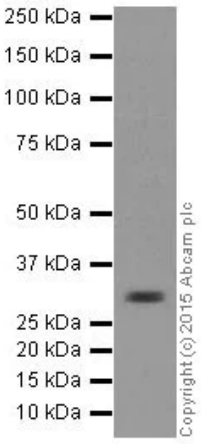
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab191881 in K562 whole cell lysate.

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

Exposure time: 5 seconds.

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

Predicted band size: 27 kDa



Western blot - Anti-Lin28B antibody [EPR18717] (ab191881)

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Lin28B antibody [EPR18717] (ab191881) at 1/2000 dilution

All lanes:
Human testis lysate at 10 µg

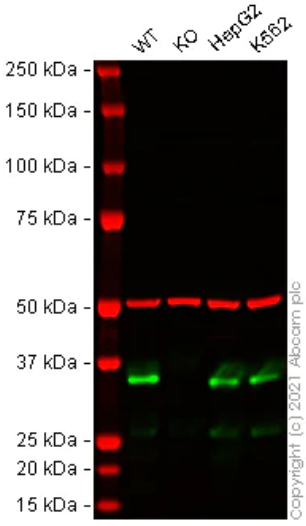
Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 27 kDa

Observed band size: 30 kDa

Exposure time: 3min



Western blot - Anti-Lin28B antibody [EPR18717] (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

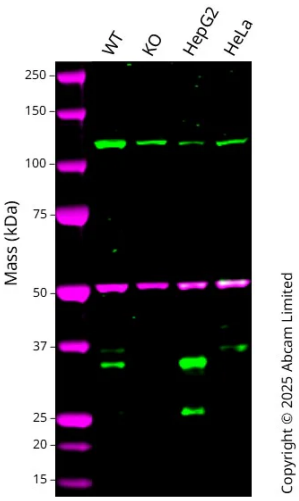
Lane 3:
HepG2 cell lysate at 20 µg

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] (ab191881)

Lin28B Western blot staining using rabbit Anti-Lin28B antibody

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

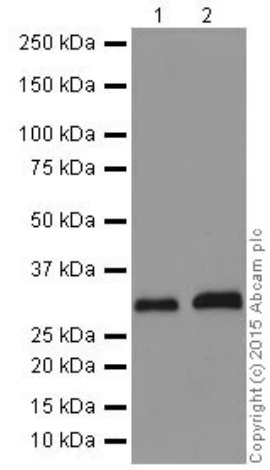
Secondary

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] (ab191881)

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Lin28B antibody [EPR18717] (ab191881) at 1/2000 dilution

Lane 1:
K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate at 20 µg

Lane 2:
HepG2 (Human liver hepatocellular carcinoma) whole cell lysate at 20 µg

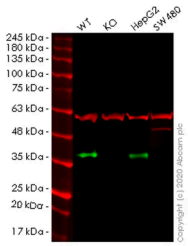
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 27 kDa

Observed band size: 30 kDa

Exposure time: 5s



Western blot - Anti-Lin28B antibody [EPR18717] (ab191881)

Lin28B Western blot staining using rabbit Anti-Lin28B antibody

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

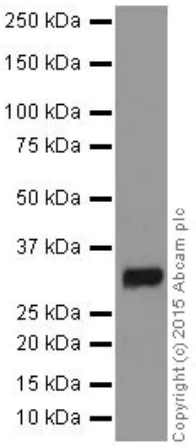
Lane 4:
SW480 cell lysate at 20 µg

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



Western blot - Anti-Lin28B antibody [EPR18717] (ab191881)

Lin28B Western blot staining of NCCIT (Human pluripotent embryonic carcinoma) whole cell lysate using rabbit Anti-Lin28B antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Lin28B antibody [EPR18717] (ab191881) at 1/2000 dilution

All lanes:
NCCIT (Human pluripotent embryonic carcinoma) whole cell lysate at 20 µg

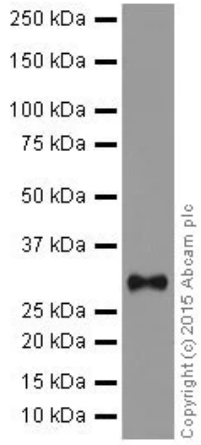
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 27 kDa

Observed band size: 30 kDa

Exposure time: 15s



Western blot - Anti-Lin28B antibody [EPR18717] (ab191881)

Lin28B Western blot staining of Human placenta lysate using rabbit Anti-Lin28B antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Lin28B antibody [EPR18717] (ab191881) at 1/2000 dilution

All lanes:
Human placenta lysate at 20 µg

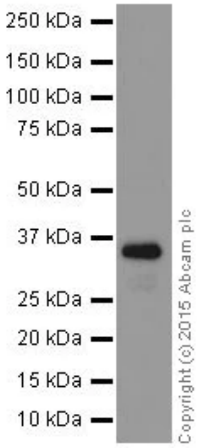
Secondary

All lanes:
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 27 kDa

Observed band size: 30 kDa

Exposure time: 3min



Western blot - Anti-Lin28B antibody [EPR18717] (ab191881)

Lin28B Western blot staining of Recombinant full length Human Lin28B protein using rabbit Anti-Lin28B antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
Recombinant Human Lin28B protein contains aa1-250 with His-Tag®.

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

All lanes:
Recombinant full length Human Lin28B protein at 0.01 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 27 kDa

Exposure time: 1s

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