

Product Datasheet

LIN-28A Antibody NBP1-49537

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NBP1-49537**LIN-28A Antibody****Product Information**

Unit Size	0.1 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Unpurified
Buffer	Whole antisera

Product Description

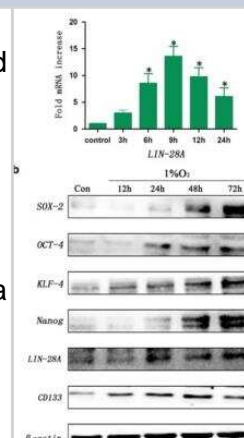
Host	Rabbit
Gene ID	79727
Gene Symbol	LIN28A
Species	Human, Mouse
Marker	Undifferentiated human embryonic stem cell Marker
Immunogen	Partial recombinant human Lin28 protein expressed in E. coli. [Swiss-Prot# Q9H9Z2]

Product Application Details

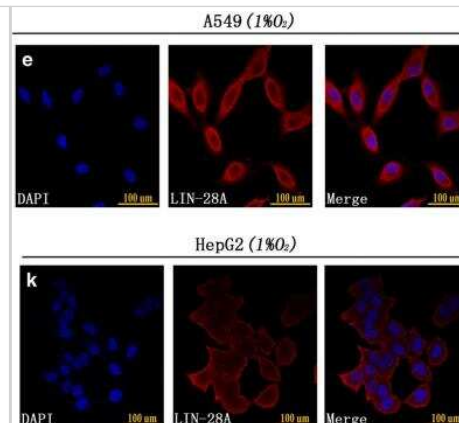
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:1000, Immunohistochemistry 1:1000, Immunocytochemistry/ Immunofluorescence 10-15 ug/ml, Immunohistochemistry-Paraffin 1:1000
Application Notes	This Lin28 antibody is useful for IHC-P, ICC/IF and Western blot.

Images

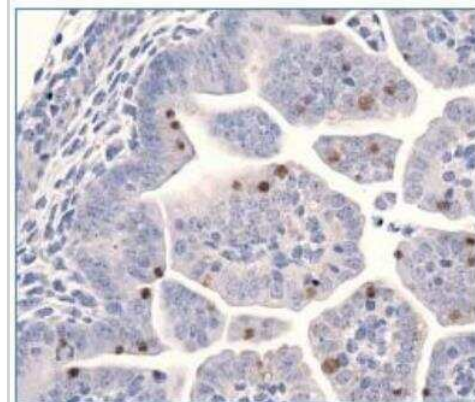
Western Blot: LIN-28A Antibody [NBP1-49537] - Hypoxia promoted an increase in the expression of SOX-2, OCT-4, KLF-4, Nanog, Lin-28A and CD133. The RT-qPCR analysis showed an upregulation in the expression of Lin-28A in a time-dependent manner in sorted A549 cells under hypoxic conditions (*P<0.05). (b) Western blot analysis showed that the transcription factors and stem cell markers were not expressed under normoxia. However, there was an increase in the expression of SOX-2 (MAB2018), OCT-4 (MAB1759), KLF-4 (AF3640), Nanog (AF1997), Lin-28A and CD133 in a time-dependent manner after hypoxia treatment in sorted A549 cells. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/cddiscovery2016105>) licensed under a CC-BY license.



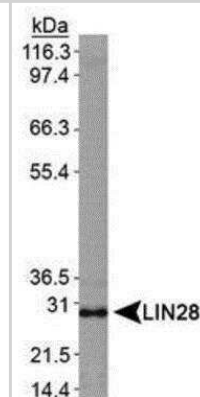
Immunocytochemistry/Immunofluorescence: LIN-28A Antibody [NBP1-49537] - (e) Immunofluorescence staining showed that A549 CD133-negative cells highly expressed Lin-28A after 48 h of 1% O₂ exposure. (k) Immunofluorescence staining showed that HepG2 CD133-negative cells highly expressed Lin-28A after 48 h of 1% O₂ exposure. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/cddiscovery2016105>) licensed under a CC-BY license.



Immunohistochemistry: LIN-28A Antibody [NBP1-49537] - Analysis of LIN28 in E17.5 mouse embryo.



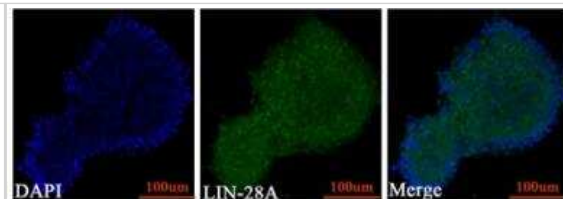
Western Blot: LIN-28A Antibody [NBP1-49537] - Analysis of LIN28 in NTERA-2 cell lysate.



Immunocytochemistry/Immunofluorescence: LIN-28A Antibody [NBP1-49537] - Analysis of LIN28 in NTERA-2 cells using NBP1-49537. Nuclei (Blue) are counterstained using Hoechst 33258.



Immunocytochemistry/Immunofluorescence: LIN-28A Antibody [NBP1-49537] - Immunofluorescence staining showed that the newly formed GL261 spheres highly expressed Lin-28A. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/cddiscovery2016105>) licensed under a CC-BY license.



Publications

B Li, W W Xu, L Han, K T Chan, S W Tsao, N P Y Lee, S Law, L Y Xu, E M Li, K W Chan, Y R Qin, X Y Guan, Q Y He, A L M Cheung MicroRNA-377 suppresses initiation and progression of esophageal cancer by inhibiting CD133 and VEGF Oncogene 2017-07-13 [PMID: 28288140]

Wang P, Wan Ww, Xiong SL et al. Cancer stem-like cells can be induced through dedifferentiation under hypoxic conditions in glioma, hepatoma and lung cancer. Cell Death Discovery. 2017-01-23 [PMID: 28179999] (WB, ICC/IF, Human)

Amen AM, Ruiz-Garzon CR, Shi J et al. A Rapid Induction Mechanism for Lin28a in Trophic Responses Mol. Cell 2017-01-20 [PMID: 28132840] (ICC/IF)

Boj SF, Hwang C, Baker LA et al. Organoid Models of Human and Mouse Ductal Pancreatic Cancer. Cell. 2014-12-31 [PMID: 25557080]

Procedures

Western Blot protocol for Lin28 Antibody (NBP1-49537)

LIN-28A Antibody:

Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH₂O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-Lin28 primary antibody (NBP1-49537) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions (Pierce ECL).

*Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunohistochemistry-Paraffin protocol for Lin28 Antibody (NBP1-49537)

LIN-28A Antibody:

Immunohistochemistry-paraffin embedded sections

Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.

Staining:

1. Wash sections in dH₂O three times for 5 minutes each.
2. Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in dH₂O.
12. Counterstain sections in hematoxylin.
13. Wash sections in dH₂O two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry/Immunofluorescence Protocol for Lin28 Antibody (NBP1-49537)

LIN-28A Antibody:

Immunocytochemistry Protocol

Culture cells to appropriate density in 35mm culture dishes or 6-well plates.

1. Pull off culture medium with and add 10% formalin to the dish. Fix at room temperature for 30 minutes..
2. Take off the formalin and add ice cold methanol (kept in well sealed bottle in -20C). Incubate for 5-10 minutes.
3. Take off methanol and add PBS (You can add 0.1% Tween-20 to PBS used here and all subsequent steps), be sure to not let the specimen dry out. Wash 3 times 10 minutes before proceeding to blocking step.
4. To block nonspecific antibody binding incubate in 10% normal goat serum for a minimum of 1 hr at room temp. Cells can also block overnight at 4C for this step.
5. Add primary antibody at appropriate dilution and incubate at room temp for 2 hrs or overnight at room temp.
6. Remove primary antibody and replace with PBS. Wash 3 x 10 min in PBS.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hr at room temperature
8. Remove antibody and replace with PBS, wash 1 x 10 min in PBS. Add Hoechst 33258 to PBS at 1:25,000 and incubate for 10 min. Wash a third time with PBS for 10 min (total of 3X10min PBS washes).
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide and parafilmed. Cells can also be coverslipped using Fluoromount. If storing coverslip be sure to seal the edges with clear nail polish.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.





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Products Related to NBP1-49537

NBP1-44968	NTERA-2 Whole Cell Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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