

# Product Datasheet

## LIN-28A Antibody (14E6-4E6)

### NBP2-22481

Unit Size: 100 ug

Store at -20C. Avoid freeze-thaw cycles.

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**NBP2-22481****LIN-28A Antibody (14E6-4E6)**

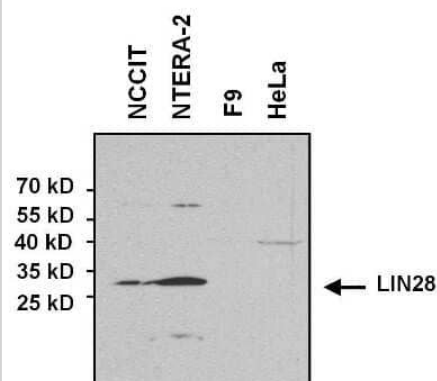
Product Information	
Unit Size	100 ug
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	14E6-4E6
Preservative	0.05% Sodium Azide
Isotype	IgG2a
Purity	Protein A purified
Buffer	PBS with 1 mg/ml BSA and 30% glycerol

Product Description	
Host	Mouse
Gene ID	79727
Gene Symbol	LIN28A
Species	Human, Mouse
Reactivity Notes	Please note that this antibody is reactive to Mouse and derived from the same host, Mouse. Additional Mouse on Mouse blocking steps may be required for IHC and ICC experiments. Please contact Technical Support for more information.
Marker	Undifferentiated human embryonic stem cell Marker
Immunogen	Full-length human recombinant protein expressed in bacteria

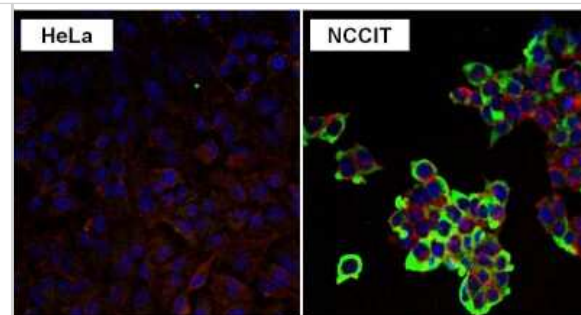
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 1:1000, Flow Cytometry 1:100, Immunohistochemistry 1:20 - 1:200, Immunocytochemistry/ Immunofluorescence 1:50 - 1:200, Immunoprecipitation 5 ug, Immunohistochemistry-Paraffin 1:20 - 1:200, Chromatin Immunoprecipitation (ChIP) 1-3 ul

**Images**

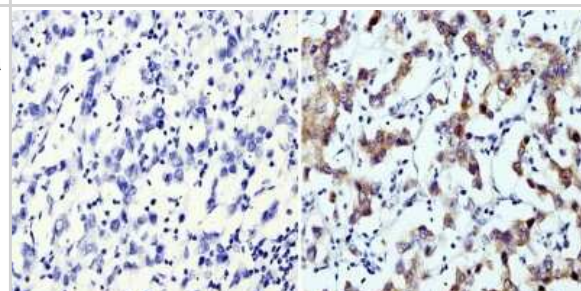
Western Blot: LIN-28A Antibody (14E6-4E6) [NBP2-22481] - Analysis of 75 ug of various whole cell lysates and 10 ul of PageRuler Prestained Protein Ladder onto a 4-20% Tris-HCl polyacrylamide gel.



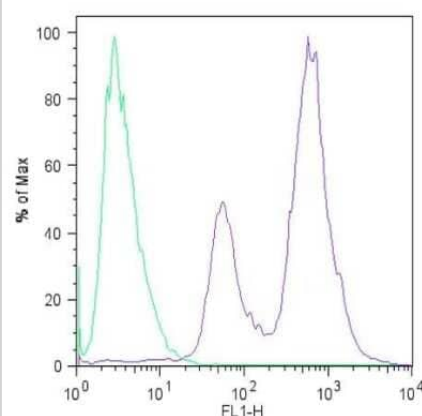
**Immunocytochemistry/Immunofluorescence:** LIN-28A Antibody (14E6-4E6) [NBP2-22481] - Analysis of LIN28 in NCCIT and HeLa cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature. Cells were blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with a LIN28 monoclonal antibody at a dilution of 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with a DyLight 488-conjugated goat anti-mouse IgG secondary antibody. F-Actin (red) was stained with DyLight-554 Phalloidin and nuclei (blue) were stained with Hoechst 33342 dye.



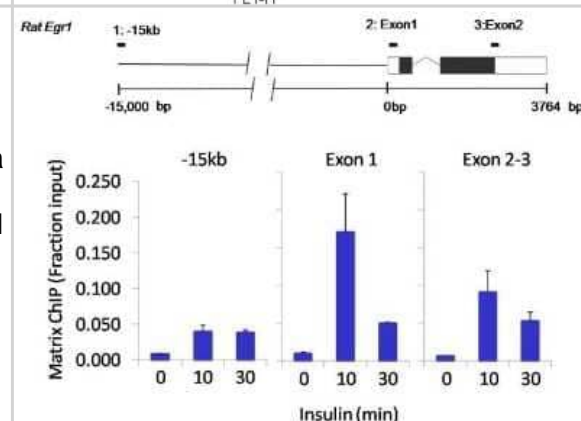
**Immunohistochemistry-Paraffin:** LIN-28A Antibody (14E6-4E6) [NBP2-22481] - Analysis showing staining in the cytoplasm of human seminoma (right) compared with a negative control without primary antibody (left).



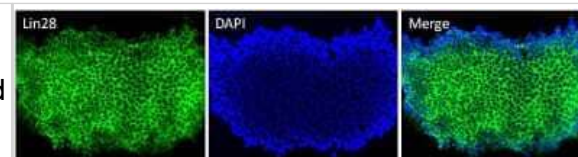
**Flow Cytometry:** LIN-28A Antibody (14E6-4E6) [NBP2-22481] - Analysis of Lin28 (blue histogram) on H9 embryonic stem cells. To generate single cells suspensions, colonies were treated with TrypLE cell dissociation enzyme for 5 minutes at 37°C. Cells were incubated with a Lin28 monoclonal antibody or mouse IgG (green histogram) at a dilution of 1:100 for 1 hour on ice, washed with PBS + 5% fetal calf serum (FACS buffer), and incubated with a fluorescein-conjugated secondary antibody at a dilution of 1:200 for 30 minutes on ice. Cells were washed with cold FACS buffer, resuspended in 500ul of FACS buffer containing 10ul of 4% paraformaldehyde.



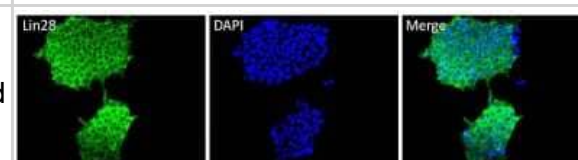
**Chromatin Immunoprecipitation:** LIN-28A Antibody (14E6-4E6) [NBP2-22481] - Analysis performed using cross-linked chromatin from rat hepatoma cells treated with insulin. IP performed using a multiplex microplate Matrix ChIP assay of LIN28 monoclonal antibody. Chromatin aliquots from cells were used per ChIP pull-down. Quantitative PCR data done in quadruplicate using 1ul of DNA in 2ul SYBR real-time PCR reactions containing primers to amplify -15kb upstream of Egr1 or exon-1 or exon-2-3 of Egr1. Quantitation of immunoprecipitated chromatin is presented as signal relative to the total amount of input chromatin. Results represent the mean  $\pm$  SEM. A schematic representation of the rat Egr-1 locus is shown; oxes represent exons (black boxes = translated, white boxes = untranslated), the zigzag line represents an intron, and the straight line represents upstream sequence. Regions amplified by Egr-1 primers are represented by black bars. Data courtesy of the Innovators Program.



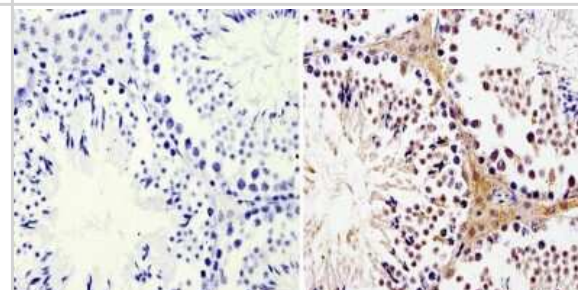
**Immunocytochemistry/Immunofluorescence: LIN-28A Antibody (14E6-4E6) [NBP2-22481]** - Analysis of Lin28 (green) in H9 embryonic stem cells grown for a few days on Matrigel-coated chamber slides. Cells fixed in 4% paraformaldehyde were permeabilized with 0.1% Triton X-100 for 15 minutes at room temperature. Cells were probed with a Lin28 monoclonal antibody at a dilution of 1:200 overnight at 4C, washed with PBST, and incubated with a fluorescein-conjugated secondary antibody at a dilution of 1:100 for 1 hour at room temperature. Nuclei (blue) were stained with DAPI and cells were analyzed by fluorescence microscopy at 20X magnification.



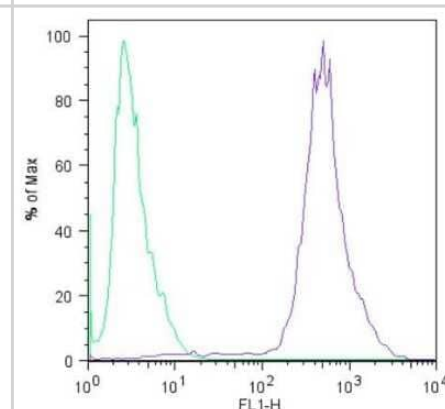
**Immunocytochemistry/Immunofluorescence: LIN-28A Antibody (14E6-4E6) [NBP2-22481]** - Analysis of Lin28 (green) in HEL 11.4 induced IPS cells grown for a few days on Matrigel-coated chamber slides. Cells fixed in 4% paraformaldehyde were permeabilized with 0.1% Triton X-100 for 15 minutes at room temperature. Cells were probed with a Lin28 monoclonal antibody at a dilution of 1:200 overnight at 4C, washed with PBST, and incubated with a fluorescein-conjugated secondary antibody at a dilution of 1:100 for 1 hour at room temperature. Nuclei (blue) were stained with DAPI and cells were analyzed by fluorescence microscopy at 20X magnification.



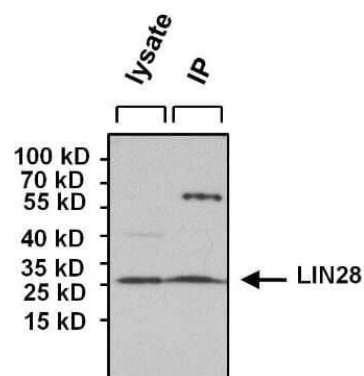
**Immunohistochemistry-Paraffin: LIN-28A Antibody (14E6-4E6) [NBP2-22481]** - Analysis showing staining in the nucleus and cytoplasm of mouse testis tissue (right) compared with a negative control without primary antibody (left).



**Flow Cytometry: LIN-28A Antibody (14E6-4E6) [NBP2-22481]** - Analysis of Lin28 (blue histogram) on HEL 11.4 induced IPS cells. To generate single cells suspensions, colonies were treated with TrypLE cell dissociation enzyme for 5 minutes at 37C. Cells were incubated with a Lin28 monoclonal antibody or mouse IgG (green histogram) at a dilution of 1:100 for 1 hour on ice, washed with PBS + 5% fetal calf serum (FACS buffer), and incubated with a fluorescein-conjugated secondary antibody at a dilution of 1:200 for 30 minutes on ice. Cells were washed with cold FACS buffer, resuspended in 500ul of FACS buffer containing 10ul of 4% paraformaldehyde.



**Immunoprecipitation: LIN-28A Antibody (14E6-4E6) [NBP2-22481] -**  
Analysis of LIN28 was performed. Antigen-antibody complexes were formed by incubating 700ug of lysate with 5 ug of an LIN28 monoclonal antibody overnight on a rocking platform at 4C. The immune complexes were captured on 50 ul Protein A/G Agarose was loaded as a positive control for detection. Samples were resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane, and blocked with 5% BSA/TBS-0.1%Tween for at least 1 hour. The membrane was probed with a LIN28 monoclonal antibody at a dilution of 1:1000 overnight rotating at 4C, washed in TBST, and probed with Clean-blot IP Detection Reagent at a dilution of 1:1000 for at least 1 hour.





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NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-96778	Mouse IgG2a Isotype Control (M2A)
NBP1-30275	Recombinant Human LIN-28A His Protein

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### **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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