Product Datasheet

CD133 Antibody
NBP2-44248SS

Unit Size: 0.025 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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Publications: 1

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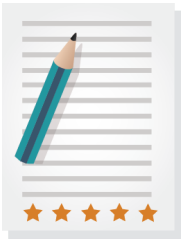
Updated 12/2/2020 v.20.1



NBP2-44248SS

CD133 Antibody

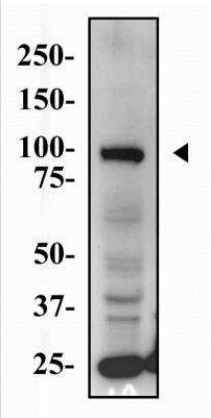
Product Information	
Unit Size	0.025 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol

Product Description	
Host	Rabbit
Gene ID	8842
Gene Symbol	PROM1
Species	Human
Reactivity Notes	This antibody is not expected to react with mouse or rat due to poor homology.
Specificity/Sensitivity	Immunogen has 100% homology to all isoforms of human CD133.
Immunogen	A synthetic peptide made to an internal portion of the human CD133 protein (between residues 600-700) [UniProt O43490]

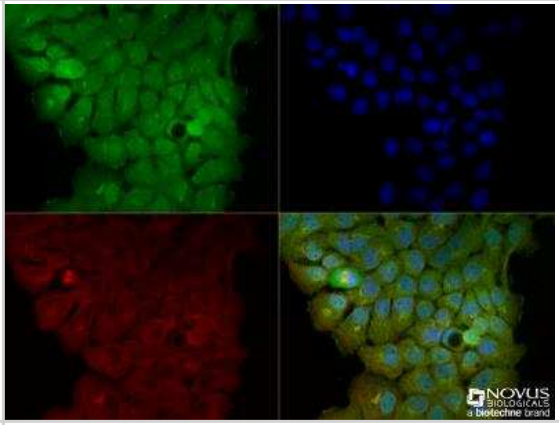
Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot 2 ug/ml, Flow Cytometry 1:1000, Immunocytochemistry/ Immunofluorescence 1:50, Flow (Intracellular) 1:1000

Images

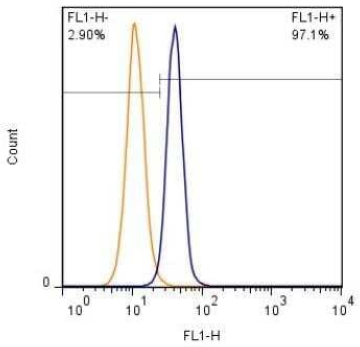
Western Blot: CD133 Antibody [NBP2-44248] - Western Blot image of anti-human CD133. Total protein from HeLa cells was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 mg/ml anti-hCD133 in 1% milk and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



Immunocytochemistry/Immunofluorescence: CD133 Antibody [NBP2-44248] - A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-hCD133 at a 1:50 dilution overnight at 4C and detected with and anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin was used as a co-stain at a 1:1000 dilution and detected with and anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Flow (Intracellular): CD133 Antibody [NBP2-44248] - Fixed and permeabilized A431 cells were stained at a 1:1000 dilution with hCD133 and detected using a Gt x ms Dylight 488 secondary (blue) shown with secondary only control (orange).



Publications

Salim EI, Aboueisha SS, Khamis AA Balanitoside as a Natural Adjuvant to Gemcitabine in Lung Cancer Experimental Model Nutrition and cancer 2022-04-12 [PMID: 35412401]

Procedures

Western Blot protocol for CD133 Antibody (NBP2-44248)
CD133 Antibody:
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 25 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute anti-CD133 primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted 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 three 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.
Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunocytochemistry/Immunofluorescence protocol for CD133 Antibody (NBP2-44248)
CD133 Antibody:
Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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

Flow Cytometry protocol for CD133 Antibody (NBP2-44248)

CD133 Antibody:

General Intracellular Cytoplasmic Target Flow Protocol

1. Grow cells to 50-75% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently with 1X PBS and scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase or Collagenase for a less damaging option.
3. Reserve 100 uL for counting, then transfer cells into a 50mL conical tube and centrifuge for 8 minutes at 400 RCF (~1200 RPM)
4. Re-suspend cells to a concentration of 1 x 106 cells/mL in Flow Buffer (1X PBS + 0.5% BSA)
5. Aliquot out 1 mL samples in accordance with your experiment.
6. Permeabilize cells by adding 100uL of a permeabiliation buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 0.5% Tween 20

b. To maintain the permeabilized state throughout your experiment, use Flow Buffer + 0.1% of the permeabilization chemical (i.e. 0.1% Tween 20 or 0.1% Saponin)

7. Following the 15 minute incubation, add 2 mL of the Flow Buffer + 0.1% permeabilizer solution to each sample.
8. Centrifuge for 5 minutes at 400 RCF (~1200 RPM)
9. Discard supernatant and re-suspend in 1 mL of Flow Buffer + 0.1% permeabilizer
10. Stain each sample at 1 uL/ 1 x 106 cells of primary antibody or 1-3 uL/ 1 x 106 cells for directly conjugated antibodies. Mix well and incubate on ice for 30 min- 1 hour. Gently mix samples every 10-15 minutes
11. Following the primary/conjugate incubation, add 2 mL/sample of Flow Buffer+0.1% permeabilizer and centrifuge for 5 min at 400 RCF (~1200RPM)
12. Remove supernatant and re-suspend each sample in 2 mL Flow Buffer+0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF (~1200 RPM)
13. The next step differs based on experimental design:

a. If using a fluorescent secondary, re-suspend the pellet in 1 mL Flow Buffer+0.1% permeabilizer and add 1 uL secondary to each sample. Mix and incubate on ice for 20 minutes.

b. If using a directly conjugated antibody, after the 2nd wash, re-suspend cell pellet to a final volume of 500 uL per sample and proceed with flow analysis.

14. In the primary-secondary experimental design, following the 20 minute secondary incubation on ice, add 1 mL Flow Buffer+0.1% permeabilizer to each sample and centrifuge for 5 minutes at 400 RCF (~1200 RPM)
15. Remove supernatant and add 2 mL Flow Buffer+0.1% permeabilizer per sample and repeat wash for 5 minutes at 400 RCF (~1200 RPM)
16. Re-suspend cell pellet to a final volume of 500 uL in Flow Buffer+0.1% permeabilizer. Proceed with flow analysis.

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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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