



CD133/1 (W6B3C1) pure human

Order no. 130-092-395

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1. Description

Clone	W6B3C1, isotype: mouse IgG1.
Product format	1 mL CD133/1 antibody, human: Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
Suggested dilution	1:100–1:200 for Western blotting (if using ECL or chromogenic substrates).
Product size	100 µg
Storage	For continuous use, store undiluted antibody conjugate at 4–8 °C for up to 6 months. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is not recommended.

1.1 Background and product applications

CD133 (also known as AC133) is a five-transmembrane domain glycoprotein that is selectively expressed on populations of hematopoietic stem and progenitor cells deriving from adult and fetal bone marrow, cord blood and peripheral blood.¹ In addition, CD133 is known to be a marker of stem cells of a variety of nonhematopoietic tissues, including neural and glial cells in the fetal brain² and neural progenitor cells in human skin³. Furthermore, CD133 was found on stem cells of prostatic epithelia⁴, muscle tissue⁵, kidney⁶, liver⁷ and corneal stroma⁸. In human fetal tissue, CD133 has been found to be expressed on the neural tube, gut and kidney⁹ and highly in fetal liver – the site of embryonic hematopoiesis. The expression of CD133 has also been demonstrated on several cell lines, for example Weri-RB-1, Y79 and Caco-2 as well as on some embryonic stem cell lines¹⁰.

The CD133/1 (W6B3C1) pure antibody represents a tool for the fast and efficient screening of CD133 protein expression in different tissues or cell lines, by Western blot or immunohistochemistry. CD133 possesses a translated sequence 95 kDa in length, though is detected at approximately 120 kDa by Western blot due to glycosylation of the protein.

Examples of applications

- Indirect detection of CD133 by Western blot analysis.
- Indirect detection of CD133⁺ cells by immunohistochemistry, immunofluorescence or flow cytometry.

2. General protocol for Western blot detection

▲ This protocol describes the transfer of proteins separated by SDS-PAGE onto a PVDF membrane in a semi-dry fashion for the subsequent detection of antibody-probed proteins by chemiluminescence. Chemiluminescent detection of peroxidase permits a 4 to 20-fold increase in detection sensitivity, though in principle chromogenic peroxidase substrates can also be used to detect immunolabeled proteins.

2.1 Instrument and reagent requirements

- Power supply (e.g. GE Healthcare EPS 601 # 18-1130-02)
- Electroblothing unit (e.g. Bio-Rad Mini Trans-Blot® Cell # 170-3930)
- Blot absorbent paper (thick) (e.g. Bio-Rad # 170-3932)
- PVDF membrane (e.g. GE Healthcare Hybond™-P # RPN303F)
- Horizontal shaker
- Glass dishes (minimum 10 × 10 cm)
- Square plastic containers (minimum 10 × 10 cm)
- Methanol
- Tris-buffered saline (TBS): 150 mM NaCl, 50 mM Tris/HCl, pH 7.6
- TBS/Tween (TBST): TBS buffer with 0.1% Tween-20
- Blocking buffer: TBST with 5% milk powder (make fresh)
- Transfer buffer: 48 mM Tris-Base, 39 mM Glycine, 1.3 mM SDS, 20% methanol.
- Polyclonal goat anti-mouse IgG-HRP (e.g. Dianova # 115-035-062)
- ECL Plus™ chemiluminescent detection reagent (GE Healthcare # RPN2132)
- (Optional) Ponceau S (Sigma Aldrich # P3504)

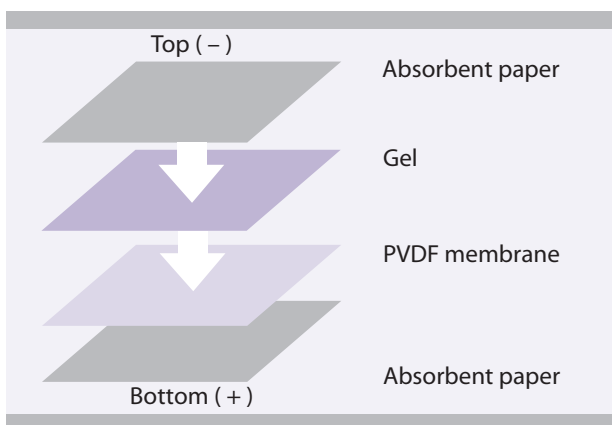


2.2 Transfer to PVDF membrane

▲ After separation of proteins by SDS-PAGE, do not stain gels for protein detection prior to electroblotting.

▲ When separating proteins for subsequent Western blot analysis, always use a pre-stained molecular weight marker in order to directly visualize bands on PVDF membrane after transfer.

1. Cut a piece of PVDF membrane (still in protective cover) to fit the size of gel from which proteins are to be transferred. Cut 2 pieces of absorbent paper to the same size.
2. Remove protective cover and soak membrane in methanol within a glass dish for at least 1 min.
3. Wash membrane in water for 30 s in a second glass dish.
4. Soak membrane in transfer buffer for 5 min, along with the absorbent paper.
5. Thoroughly clean transfer cassette of electroblotting module with water before constructing the following layers within:



▲ **Note:** Be sure to orientate layers within cassette correctly. Failure to do so will result in no transfer of proteins to the PVDF membrane. Membrane must always be placed on the side of the gel closest to positive (+) electrode.

6. Remove trapped air bubbles by rolling out with, for example a clean glass pipette. Pour off excessive transfer buffer.

▲ **Note:** Failure to remove air bubbles can lead to areas with no protein transfer, which may interfere with downstream staining performance.

7. Close lid on apparatus and connect to power supply. Apply power using the recommended settings 25 V, 100 mA, 5 W continuously for 40 min, or at user-optimized settings when electroblotting unit differs from that listed above.

8. Afterwards, disassemble layers and rinse membrane briefly with TBS in a glass dish. Discard gel and absorbent paper.

9. Mark bands of pre-stained molecular weight marker with a pen.

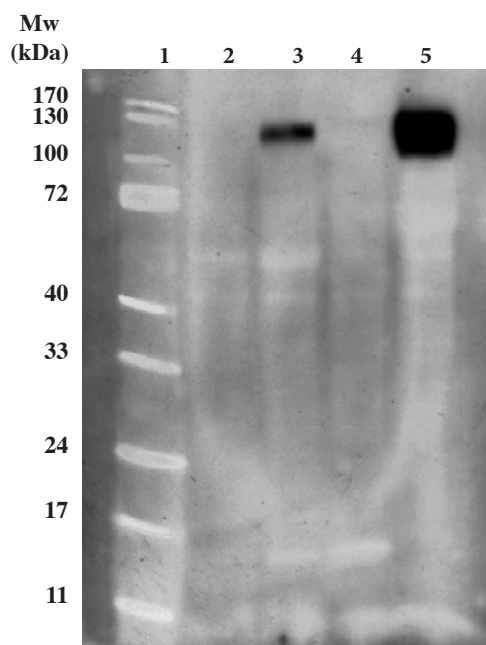
▲ **Note:** (Optional) Performance of transfer can be controlled by reversibly staining the membrane directly with Ponceau S. For details, see protocol given by the manufacturer.

10. Incubate PVDF membrane overnight in blocking buffer at 4 °C.

2.3 Chemiluminescent detection of CD133

1. Pour off blocking buffer. Wash PVDF membrane 3 × 5 min with TBST. Pour off TBST.
2. Dilute 50–100 µL of CD133/1 (W6B3C1) pure antibody in 10 mL blocking buffer. Apply to membrane and incubate on horizontal shaker at room temperature for 60 min.
3. Wash membrane 3 × 5 min with TBST. Pour off TBST.
4. Dilute secondary antibody (goat anti-mouse IgG-HRP) to the appropriate working concentration in 10 mL blocking buffer. Apply to membrane and incubate on horizontal shaker at room temperature for 60 min.
5. Wash membrane 3 × 5 min with TBST. Pour off TBST.
6. Follow manufacturer's instructions for detection of CD133 protein with ECL Plus detection reagent by chemiluminescence.

3. Example of Western blot using CD133/1 (W6B3C1) pure antibody



Protein extracts of human hippocampus (Lane 2), human fetal brain (Lane 3) and Weri-Rb-1 cells (Lane 5) were blotted onto a PVDF membrane and probed with CD133/1 (W6B3C1) pure antibody. Lane 4 is empty. Goat anti-mouse IgG-HRP was applied at a dilution of 1:10,000 before bands were developed using ECL Plus chemiluminescent detection reagent. The molecular weight marker (Lane 1) indicates CD133 reactivity at approximately 120 kDa in Lanes 3 and 5.

4. References

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Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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