Immunohistochemical detection of CD133
human

Special protocol

Index

1. Description

1.1 Background

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 brain[2] and neural progenitor cells in human skin[3]. Furthermore, CD133 was found on stem cells of prostatic epithelia[4], muscle tissue[5], kidney[6], liver[7], and corneal stroma[8]. CD133 has also been found to be expressed on cancer stem cells of a variety of cellular and tissue origin.[9] In human fetal tissue, CD133 has been found to be expressed on the neural tube, gut, and kidney[10] and highly in fetal liver – the site of embryonic hematopoiesis.

1.2 Reagent and instrument requirements

- 100% ethanol
- 100% methanol
- ChemMate™ Target Retrieval Solution (10×) (DakoCytomation, # S 2031)
- 30% (w/w) Hydrogen peroxide solution (H_2O_2)
- CD133/1 (AC133) pure, human (Miltenyi Biotec, # 130-090-422)
- VECTASTAIN® ABC kit (Mouse IgG) (Vector Laboratories, # PK-4002)

  ▲ **Note:** The VECTASTAIN ABC kit contains the following reagents: blocking serum, biotinylated affinity-purified anti-mouse immunoglobulin antibody, Reagent A (Avidin DH solution), and Reagent B (biotinylated horseradish peroxidase A).
- 3,3'-Diaminobenzidine tetrahydrochloride (DAB) (Sigma, # D5637)
- Nickel(II) chloride ($NiCl_2$)
- Tris base
- Sodium chloride (NaCl)
- Hydrochloric acid (HCl)
- Methyl green solution or Meyer's hemalum
- Phosphate-buffered saline (PBS)
- Deionized water
- Tap water
- H_2O_2
- Water bath
- Microwave
- Fluorescence microscope
- Hydrophobic pen
- Cover slides of appropriate length
- Hellendahl jars
- Plastic Hellendahl jars
- Humidified chamber
- Roti®-Histol (Roth, # 6640.1)
- Roti®-Histokitt (Roth, # 6638.1)

1.3 Reagent preparation

Prepare the following stock solutions:

- Ethanol dilution series: 100%, 96%, 80%, 70% ethanol in deionized water. Store at room temperature.
- 10× Tris buffer: Dissolve 61 g of Tris base and 116.9 g of NaCl in 1 L of deionized water. Adjust pH to 7.6 (±0.1) with HCl. Store at room temperature.
- DAB stock solution: Dissolve 5 g of DAB tetrahydrochloride in 132 mL of 1× Tris buffer. Prepare 4 mL aliquots and store at –20 °C.

Prepare the following working solutions:

▲ **Note:** Always prepare fresh working solutions.

- Target Retrieval Solution: Dilute 10× Target Retrieval Solution 1:10 with deionized water.
- 3% H_2O_2: Dilute 30% H_2O_2 solution 1:10 with 100% methanol.
- VECTASTAIN ABC kit: Prepare reagents according to the manufacturer's instructions.
- $NiCl_2$ working solution: Dissolve 8 g of $NiCl_2$ in 100 mL of deionized water. Store at 4 °C.

  ▲ **Note:** Caution! $NiCl_2$ is toxic.
- DAB tetrahydrochloride working solution: Dilute 4 mL of the DAB stock solution with 175 mL of 1× Tris buffer. Warm to 37 °C in a water bath.

2. Protocol for immunohistochemical staining of paraffin-embedded tissue sections

1. Prepare sections of Paraffin-embedded tissues of no more than 2 µm in thickness.

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2. Deparaffinize and rehydrate the tissue sections by serial immersion in Hellendahl jars containing the following:

   2 × 5 min Roti-Histol

   2 × 2 min 100% ethanol

   2 × 2 min 96% ethanol

   2 × 2 min 80% ethanol

   2 × 2 min 70% ethanol

   1 × 5 min PBS

   1 × brief rinse with deionized water

3. For antigen retrieval, fill a plastic Hellendahl jar with 1× Target Retrieval Solution and submerge the tissue sections completely in the solution. Heat uncovered at 250–300 W in a microwave for 15 minutes. Replace any evaporated Target Retrieval Solution and leave to cool to room temperature.

4. Wash 1 × 5 minutes with deionized water, followed by 1 × 5 minutes with PBS in a Hellendahl jar.

5. Block endogenous peroxidase activity by immersing slide in 3% H_2O_2 solution for 30 min at room temperature.

   ▲ **Note:** For tissue sections with low endogenous peroxide activity, e.g. organ tissue, the incubation time can be shortened to 10 minutes.

6. Wash 2 × 3 minutes with fresh PBS in a Hellendahl jar.

7. Dab slide dry around the section and encircle it with a hydrophobic pen.

8. Block non-specific binding of CD133/1 (AC133) pure antibody by incubating slide in a humidified chamber at room temperature for 20 minutes in the VECTASTAIN ABC kit blocking serum.

9. Drain off supernatant by tilting the slide and then blot dry. Do not wash the slide.

10. Pipette 50–250 µL of CD133/1 (AC133) pure antibody, diluted in the VECTASTAIN ABC kit blocking serum, to each section and incubate slide for 1 hour at room temperature in a humidified chamber.

    ▲ **Note:** The optimal working concentration of antibody should be pre-determined as it will vary according to tissue type and thickness of sections.

    ▲ **Note:** For a negative control, use VECTASTAIN ABC kit blocking serum only.

11. Wash 2 × 3 minutes with fresh PBS in a Hellendahl jar.

12. Pipette carefully 50–250 µL of the VECTASTAIN ABC kit biotinylated secondary antibody, diluted in the blocking serum, to each section and incubate slide for 30 minutes at room temperature in a humidified chamber.

    ▲ **Note:** During this incubation step, prepare the VECTASTAIN ABC reagent according to the manufacturer's instructions. This reagent must be incubated at room temperature for 30 minutes before use.

13. Wash 2 × 3 minutes with fresh PBS in a Hellendahl jar.

14. Pipette carefully 50–250 µL of freshly prepared VECTASTAIN ABC reagent to each section. Incubate for 30 minutes at room temperature in a humidified chamber.

15. Wash 2 × 3 minutes with fresh PBS in a Hellendahl jar.

16. Add 0.1 mL of the 3% H_2O_2 working solution and 1 mL of the $NiCl_2$ working solution to the pre-warmed DAB tetrahydrochloride working solution.

    ▲ **Note:** Caution! $NiCl_2$ is toxic.

17. Incubate tissue sections in the DAB working solution in a Hellendahl jar for 10 minutes at 37 °C in the water bath.

18. Wash 2 × 3 minutes with tap water.

19. Counterstain sections by incubating for 1–2 minutes at room temperature in Methyl Green solution or Meyer's hemalum. Shake occasionally.

20. Dehydrate the tissue sections by serial immersion in Hellendahl jars containing the following:

    2 × brief immersion in 96% ethanol

    3 × brief immersion in 100% ethanol

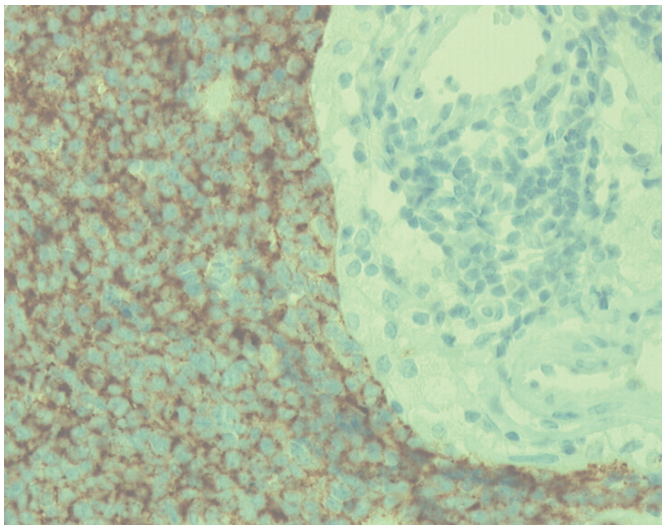
    ▲ **Note:** Last ethanol step must be clear after immersion of the tissue sections.

    2 × 5 min Roti-Histol

21. Mount sections directly using the Roti-Histokitt and apply coverslip.

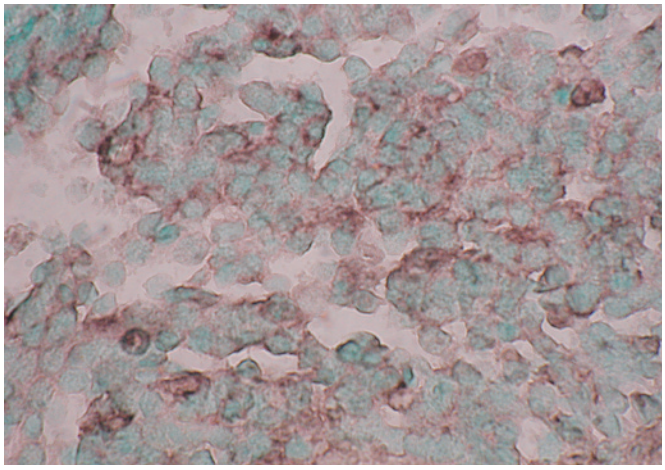
3. Example immunohistochemical staining for CD133

A. Paraffin-embedded tissue sections of a human Pineoblastoma were stained with CD133/1 (AC133) pure and developed using DAB as the substrate (brown color). Hemalum was used for counterstaining.



Courtesy of Dr. Michel Mittelbronn, Universitiy of Tübingen, Germany.

B. Paraffin-embedded tissue sections of a human Pineoblastoma were stained with CD133/1 (AC133) pure and developed using the VECTASTAIN ABC kit with DAB as the substrate (brown color). Methyl Green was used for counterstaining.



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