Miltenyi Biotec

Immunofluorescence staining with Anti-Myosin Heavy Chain, Anti-MLC2v, or Anti-MLC2a antibodies for microscopy

Contents

1. Description

1.1 Background information

This protocol describes a simplified and fast protocol for the immunofluorescent staining of cultivated cardiomyocytes as well as human, mouse, or rat heart tissue for fluorescence microscopy. Recombinantly engineered REAfinity™ Antibodies enable the unambiguous analysis of cardiomyocytes.

1.2 Reagent and instrument requirements

- Anti-Myosin Heavy Chain pure, human, mouse, rat (# 130-112-757), Anti-MLC2v pure, human, mouse, rat (# 130-112-759), or Anti-MLC2a pure, human, mouse, rat (# 130-112-758)
- Phosphate-buffered saline (PBS)
- autoMACS® Running Buffer (# 130-091-221)
- Secondary antibody, e.g., Anti-IgG (H+L)-Vio® 515, human (# 130-112-760)

Additional requirements when working with cultivated cells (refer to protocol 2.1)

- Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells containing Inside Fix and Inside Perm.

Additional requirements for acetone-fixed tissue cryosections (refer to protocol 2.2)

- Tissue freezing medium
- Cryostat
- Adhesion slides and cover slips
- Icecold acetone
- Cryomolds
- Distilled water
- Fluorescence mounting medium

Additional requirements for formalin-fixed paraffin-embedded (FFPE) tissue sections (refer to protocol 2.3)

- Inside Fix of the Inside Stain Kit (# 130-090-477) for the fixation of tissue.
- 100% Ethanol
- Xylol
- Embedding cassettes
- Embedding cassettes, large
- Metal embedding mold
- Paraffin

  ▲ **Note:** Melt paraffin overnight at 60 °C before use.
- Microtome
- Adhesion slides and cover slips
- Distilled water
- When working with Anti-Myosin Heavy Chain or Anti-MLC2v antibodies:
  TEC buffer: Prepare a stock solution by dissolving 2.5 g TRIS base, 5 g EDTA, and 3.2 g tri-sodium citrate dihydrate in 1 L distilled water. Adjust to pH 9 with 10 M NaOH. Dilute 1:10 for ready-to-use solution. Pre-heat at 95 °C for 20 minutes before use.

  When working with Anti-MLC2a antibodies:
  Citrate buffer: Prepare a stock solution by dissolving 29.4 g tri-sodium citrate dihydrate in 850 mL distilled water. Adjust to pH 6 with 0.1 M citrate acid (approximately 15 mL) and fill up to 1 L. Dilute 1:10 for ready-to-use solution, revise pH 6.
- Sudan Black solution: Dissolve 0.3% Sudan Black B in 70% ethanol and mix for 2 hours in the dark.
- Fluorescence mounting medium
- (Optional) MACSmix™ Tube Rotator (# 130-090-753)

2. Protocol

2.1 Protocol when working with cultivated cells

2.1.1 Cultivated cell preparation

1. Wash cells 2× with PBS.
2. Dilute Inside Fix (Inside Stain Kit) 1:1 with PBS.
3. Fix cells with Inside Fix for 10 minutes in the dark at room temperature.
4. Wash cells 2× with PBS.

   ▲ **Note:** Fixed cells can be stored in azide-containing buffer at 2–8 °C for up to 1 week.

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2.1.2 Staining

1. Add the Anti-Myosin Heavy Chain pure, Anti-MLC2v pure, or Anti-MLC2a pure antibody 1:50 to Inside Perm, e.g., 2 µL antibodies to 98 µL Inside Perm, and add the solution to the cells.
2. Incubate for 10 minutes in the dark at room temperature.
3. Wash cells 2× with PBS.
4. Add an appropriate secondary antibody, e.g., Anti-IgG (H+L)-Vio® 515, 1:100 to Inside Perm and add the solution to the cells.
   ▲ **Note:** Add, if using, a cell nucleus marker, e.g., DAPI, also in step 8.
5. Incubate for 10 minutes in the dark at room temperature.
6. Wash cells 2× with PBS.
7. Cells are now ready for immunofluorescence microscopy.
   ▲ **Note:** Samples can be stored at 2–8 °C in the dark for up to one week.

2.2 Protocol for acetone-fixed tissue cryosections

2.2.1 Tissue preparation

1. Cover cryomold with tissue freezing medium.
2. Add adult mouse or rat heart to the tissue freezing medium.
3. Fill up the cryomold with tissue freezing medium and freeze samples on dry ice or by using isopentane and liquid nitrogen.
   ▲ **Note:** Embedded tissue can be stored at –80 °C until use.
4. Prepare 4–6 µm slices using a cryostat.
5. Transfer slices onto adhesion slides.
   ▲ **Note:** Slides may be stored at –80 °C for up to one year.
6. Fix slices with icecold acetone for 3 minutes.
7. Block unspecific binding by adding slides into autoMACS® Running Buffer for 15 minutes at room temperature.

2.2.2 Staining

1. Add the Anti-Myosin Heavy Chain pure, Anti-MLC2v pure, or Anti-MLC2a pure antibody 1:50 to autoMACS Running Buffer, e.g., 2 µL antibodies to 98 µL autoMACS Running Buffer, and add the solution to the slices.
2. Incubate within a moistured chamber for 10 minutes at room temperature.
   ▲ **Note:** Assure that slices do not dry out.
3. Wash slices 3× with autoMACS Running Buffer.
4. Add an appropriate secondary antibody, e.g., Anti-IgG (H+L)-Vio515, 1:100 to autoMACS Running Buffer and add the solution to the cells.
   ▲ **Note:** Add, if using, a cell nucleus marker, e.g., DAPI, also in step 4.
5. Incubate for 10 minutes in the dark at room temperature.
6. Wash slices 3× with autoMACS Running Buffer.
7. Wash slices 1× with distilled water.
8. Place one drop of fluorescence mounting medium onto each slice.
9. Cover with cover slips, avoid air bubbles.
10. Store for 30 minutes at room temperature.
11. Store overnight at 2–8 °C.
12. Slides are now ready for immunofluorescence microscopy.
    ▲ **Note:** Slides can be stored at 2–8 °C in the dark for up to one month.

2.3 Protocol for formalin-fixed paraffin-embedded (FFPE) tissue sections

2.3.1 Tissue preparation

1. Transfer heart tissue to Inside Fix Solution (Inside Stain Kit) and incubate in the dark overnight at room temperature under continuous rotation by using, e.g., the MACSmix™ Tube Rotator.
2. Transfer tissue to distilled water and incubate for 15 minutes at room temperature. Repeat this step.
3. Transfer tissue to 70% ethanol and incubate for 45 minutes at room temperature. Repeat this step.
4. Transfer tissue to 80% ethanol and incubate for 45 minutes at room temperature. Repeat this step.
   ▲ **Note:** The second incubation step can be overnight.
5. Transfer tissue to 90% ethanol and incubate for 45 minutes at room temperature. Repeat this step.
6. Transfer tissue to 96% ethanol and incubate for 45 minutes at room temperature. Repeat this step.
7. Transfer tissue to xylol in a large embedding cassette and incubate for 2 hours at room temperature. Repeat this step.
8. Transfer tissue to melted paraffin and incubate for 2 hours at 60 °C.
9. Transfer tissue to fresh, melted paraffin and incubate for 2 hours at 60 °C.
   ▲ **Note:** Samples can also be stored overnight.
10. Transfer tissue to fresh, melted paraffin and incubate for 2 hours at 60 °C.
11. Add melted paraffin into a metal embedding mold until the bottom is covered.
12. Place the tissue into the mold.
13. Place mold on ice and carefully push the tissue into the paraffin.
14. Place the bottom of a normal embedding cassette onto the top of the mold.
15. Fill up with paraffin until the end of the embedding cassette is reached.
16. Cool down on ice and remove cassette with embedded tissue from the mold.
    ▲ **Note:** Samples can be stored at room temperature until use.
17. Prepare 3–6 µm slices using a microtome and place each slice on a slide.
    ▲ **Note:** Assure that slices are completely spread out on the slides. Straighten slices in a warm water bath at 37 °C.
18. Incubate overnight at 40 °C.
19. Transfer slides to xylol and incubate for 20 minutes at room temperature under continuous movement.

20. Transfer slides to 100% ethanol and incubate for 1 minute at room temperature under continuous movement.
21. Transfer slides to 95% ethanol and incubate for 1 minute at room temperature under continuous movement.
22. Transfer slides to 80% ethanol and incubate for 1 minute at room temperature under continuous movement.
23. Transfer slides to 70% ethanol and incubate for 1 minute at room temperature under continuous movement.
24. Transfer slides to 50% ethanol and incubate for 1 minute at room temperature under continuous movement.
25. Transfer slides to distilled water and incubate for 1 minute at room temperature under continuous movement. Repeat this step.
26. Transfer slides to 500–1000 mL pre-heated TEC buffer (Anti-Myosin Heavy Chain or Anti-MLC2v antibodies) or citrate buffer (Anti-MLC2a antibody) and boil at 95 °C for 20 minutes.
27. Cool down for maximal 20 minutes.
28. Wash 1× with autoMACS® Running Buffer.
29. Incubate with Sudan Black solution for 10 minutes at room temperature.
30. Wash 8× with autoMACS Running Buffer.

2.3.2 Staining

1. Add the Anti-Myosin Heavy Chain pure, Anti-MLC2v pure, or Anti-MLC2a pure antibody 1:50 to autoMACS Running Buffer, e.g., 2 µL antibodies to 98 µL autoMACS Running Buffer, and add the solution to the slices.
2. Incubate within a moistured chamber for 10 minutes at room temperature.
   ▲ **Note:** Assure that slices not dry out.
3. Wash 3× with autoMACS Running Buffer.
4. Add an appropriate secondary antibody, e.g., Anti-IgG (H+L)-Vio® 515, 1:100 to autoMACS Running Buffer and add the solution to the slices.
   ▲ **Note:** Add, if using, a cell nucleus marker, e.g., DAPI, also in step 4.
5. Incubate for 10 minutes in the dark at room temperature.
6. Wash 3× with autoMACS Running Buffer.
7. Wash 1× with distilled water.
8. Place one drop of fluorescence mounting medium onto each slice.
9. Cover with cover slips, avoid air bubbles.
10. Store for 30 minutes at room temperature.
11. Store overnight at 2–8 °C.
12. Slides are now ready for immunofluorescence microscopy.
    ▲ **Note:** Slides can be stored at 2–8 °C in the dark for up to one month.

For examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols.