Miltenyi Biotec

Immunofluorescence staining with neural cell antibodies for microscopy

Contents

1. Description

1.1 Background information

This protocol describes a simplified and fast protocol for the immunofluorescent staining of both cultivated and tissue neural cells, including astrocytes, microglia, oligodendrocytes, and neuronal cells for fluorescence microscopy.

1.2 Reagent and instrument requirements

- Anti-ACSA-2 pure, mouse (# 130-099-138), Anti-GLAST (ACSA-1) pure, human, mouse, rat (# 130-095-822), Anti-O4 pure, human, mouse, rat (# 130-115-810), Anti-PSA-NCAM pure, human, mouse, rat (# 130-115-809), CD11b pure, human and mouse (# 130-115-811), CD68 pure, mouse (# 130-115-808), or CD171 (L1CAM) pure, mouse (# 130-115-812)

  ▲ **Note:** Store antibodies in aliquots at –20 °C. To avoid repeated freeze-thaw cycles prepare working aliquots.

- Staining buffer: Prepare a solution containing autoMACS® Running Buffer (# 130-091-221) with FcR Blocking Reagent, mouse (# 130-092-575) or human (# 130-059-901) (1:10).
- Phosphate-buffered saline (PBS)
- autoMACS Running Buffer (# 130-091-221)
- (Optional) 0.2% TRITON™ X-100 in PBS
- Corresponding secondary antibody (refer to table 1)
- Distilled water

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

- 2% paraformaldehyde (PFA) for the fixation.
- (Optional) Imaging Plate CG 1.5 (24 well) (# 130-098-263)

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

- Tissue freezing medium
- Cryostat
- Cryomolds
- Dry ice or isopentane and liquid nitrogen
- Adhesion slides and coverslips
- Fluorescence mounting medium

2. Protocols

2.1 Staining of cultivated adherent cells

1. Wash cells 3× with PBS.
2. Fix cells with 2% PFA for 10 minutes at room temperature.
3. Wash cells 3× with PBS.

   ▲ **Note:** When working with CD68 antibodies, add 0.2% TRITON X-100 in PBS, incubate for 10 minutes at room temperature, and wash cells 3× with autoMACS Running Buffer. Do not treat cells with TRITON X-100 before staining with Anti-ACSA-2, Anti-O4, or Anti-PSA-NCAM antibodies.

   ▲ **Note:** Fixed cells can be stored in azide-containing buffer at 2–8 °C for up to 1 week.
4. Add staining buffer and incubate for 10 minutes at room temperature.
5. Discard staining buffer.
6. Add pure antibody of choice in staining buffer to the cells and incubate in the dark. For incubation temperature and time as well as recommended antibody concentration refer to table 1.
7. Wash cells 3× with autoMACS Running Buffer.
8. Add a corresponding secondary antibody in staining buffer to the cells and incubate in the dark. For incubation temperature and time as well as recommended antibody concentration refer to table 1.
9. Wash cells 3× with autoMACS Running Buffer.

   ▲ **Note:** For co-staining with additional antibodies repeat step 6–9.
10. Store cells in autoMACS Running Buffer.
11. Cells are now ready for immunofluorescence microscopy.

    ▲ **Note:** Samples can be stored at 2–8 °C in the dark for up to one week.

    ▲ **Note:** When working with cells cultured on coverslips, the coverslips need to be mounted onto slides before imaging.

2.2 Staining of PFA-fixed tissue cryosection

1. Prepare PFA-fixed cryoprotected neural tissue.

   ▲ **Note:** PFA-perfused cryoprotected neural tissue can also be used.
2. Embed the tissue in tissue freezing medium and freeze the tissue on dry ice or by using isopentane and liquid nitrogen.

   ▲ **Note:** Embedded tissue can be stored at –80 °C until use.
3. Prepare approximately 10 µm slices using a cryostat.
4. Transfer slices onto adhesion slides.

SP0100.01

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

5. Wash slices carefully 3× with PBS.

▲ **Note:** Avoid pipetting directly onto the slice.

▲ **Note:** When working with CD68 antibodies, add 0.2% TRITON™ X-100 in PBS, incubate for 10 minutes at room temperature, and wash cells 3× with autoMACS® Running Buffer. Do not treat cells with TRITON X-100 before staining with Anti-ACSA-2, Anti-O4, or Anti-PSA-NCAM antibodies.

6. Add staining buffer and incubate for 10 minutes at room temperature.
7. Carefully discard staining buffer completely.
8. Add pure antibody of choice in staining buffer to the slices and incubate in the dark. For incubation temperature and time as well as recommended antibody concentration refer to table 1.
9. Wash slices 3× with autoMACS Running Buffer.
10. Add a corresponding secondary antibody in staining buffer to the slices and incubate in the dark. For incubation temperature and time as well as recommended antibody concentration refer to table 1.
11. Wash slices 3× with autoMACS Running Buffer.

▲ **Note:** For co-staining with additional antibodies repeat step 8–11.

12. Wash slices 1× with distilled water to remove salts.
13. Place one drop of fluorescence mounting medium onto each slice.
14. Cover with coverslips, avoid air bubbles.
15. Store for at least 1 hour at room temperature.
16. Store overnight at 2–8 °C.
17. Slices are now ready for immunofluorescence microscopy.

▲ **Note:** Slides can be stored at 2–8 °C in the dark for up to one month.

3. References

1. Jungblut, M. *et al.* (2012) Isolation and characterization of living primary astroglial cells using the new GLAST-specific monoclonal antibody ACSA-1. Glia 60 (6): 894–907.
2. G. Kantzer, C. *et al.* (2017) Anti-ACSA-2 defines a novel monoclonal antibody for prospective isolation of living neonatal and adult astrocytes. Glia 65 (6): 990–1004.
3. Batiuk, M. Y. *et al.* (2017) An immunoaffinity-based method for isolating ultrapure adult astrocytes based on ATP1B2 targeting by the ACSA-2 antibody. J. Biol. Chem. 292 (21): 8874–8891.

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

Antibody	Incubation temperature primary antibody	Incubation time primary antibody	Recommended antibody concentration	Secondary antibody	Incubation temperature secondary antibody	Incubation time secondary antibody
Anti-ACSA-2	Room temperature	10 minutes	1–5 µg/mL	Anti-rat IgG2b	Room temperature	10 minutes
Anti-GLAST (ACSA-1)	Room temperature	10 minutes	1–5 µg/mL	Anti-mouse IgG2a	Room temperature	10 minutes
Anti-O4	Room temperature	10 minutes	5–10 µg/mL	Anti-mouse IgM	Room temperature	10 minutes
Anti-PSA-NCAM	Room temperature	10 minutes	5–10 µg/mL	Anti-mouse IgM	Room temperature	10 minutes
CD11b	Room temperature	10 minutes	5–10 µg/mL	Anti-rat IgG2bκ	Room temperature	10 minutes
CD68	2–8 °C	Overnight	5–10 µg/mL	Anti-rat IgG2a	Room temperature	1 hour
CD171 (L1CAM)	2–8 °C	Overnight	5–10 µg/mL	Anti-rat IgG2a	Room temperature	1 hour

Table 1: Overview of incubation temperatures and times as well as recommended antibody concentrations.