MACSima™ Imaging Cyclic Staining (MICS)
Sample preparation protocol for frozen tissue

Miltenyi Biotec

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Overview

This sample preparation protocol for MACSima Imaging Cyclic Staining (MICS) is similar to standard immunohistochemistry techniques in terms of tissue collection and tissue processing. Note that this protocol is used for testing of Miltenyi Biotec's MICS antibodies but may not be optimized for all antibodies or tissue types.

1. Tissue sectioning using a cryostat

Material:

- Frozen tissue sample embedded in OCT compound. To be prepared according to standard immunohistochemistry protocols.
- Superfrost® Plus Slides (VWR, # 631-9483)
- Positioning template supplied with the MACSwell Imaging Frames: MACSwell™ One Imaging Frames (Miltenyi Biotec, # 130-124-673), MACSwell One Small Imaging Frames (Miltenyi Biotec, # 130-126-794), MACSwell Two Imaging Frames (Miltenyi Biotec, # 130-124-675), MACSwell Four Imaging Frames (Miltenyi Biotec, # 130-124-676)
- Cryostat (e.g. LEICA, # CM1860 UV)

- Place embedded tissue sample to be sectioned in the chamber of the cryostat to bring it to temperature (if tissue was stored at –80 °C equilibrate it in the chamber for at least 30 minutes prior to sectioning).
- Cut 8 µm thick sections
- Mount the required number of sections (depending on MACSwell format) onto a room temperature slide. The positioning template shows the optimal position of the section.
- Keep the slide(s) in the cryostat or on dry ice before performing next steps
- For fixation with acetone, directly continue with 2.2 Acetone fixation
- For fixation with PFA, store at -80 °C until further processing.

2. Tissue fixation

After sectioning, tissue sections can be fixed using either PFA or acetone. Please note that due to intrinsic differences in fixation mechanisms the choice of fixation method has impact on the performance of individual antibodies for immunostaining as well as on quality of tissue morphology preservation. Before fixing the sample please verify that the antibodies of interest are validated for the given fixation method.

Acetone fixation does not chemically crosslink sample molecules but precipitates proteins on the cellular architecture thereby representing a less stringent fixation method as compared to fixation with PFA. Depending on the tissue type, acetone-fixed samples can show changes in tissue morphology upon prolonged incubation times in buffer or long-cycle MICS experiments (> 30 cycles). It is recommended to test the compatibility of the fixation method with the target tissue for the application of interest prior to MICS experiments.

2.1 Paraformaldehyde (PFA) fixation

Material:

- Slide with frozen non-fixed tissue section (stored at -80 °C)
- Paraformaldehyde (PFA) solution 4 % (Fisher Scientific, # 15670799 or Biotrend, # AR1068)
- MACSima Running Buffer (Miltenyi Biotec, # 130-121-565)
- MACSwell One Imaging Frames (# 130-124-673), MACSwell One Small Imaging Frames (Miltenyi Biotec, # 130-126-794), MACSwell Two Imaging Frames (# 130-124-675), MACSwell Four Imaging Frames (# 130-124-676)

- Remove the slide with frozen non-fixed tissue from freezer.
- Proceed immediately with mounting the selected MACSwell Imaging Frame on the slide (as described in the MACSwell Imaging Frames datasheet)
- Add the appropriate volume of 4 % PFA solution according to the MACSwell Imaging Frame
    - 1000 µL per well of a MACSwell One
    - 500 µL per well of a MACSwell Two or MACSwell One Small

    - 250 µL per well of a MACSwell Four Imaging Frame
- Incubate for 10 min at room temperature.
- Wash the slide two times with MACSima Running Buffer depending on the used MACSwell Imaging Frame:
    - 1900 µL per well of a MACSwell One
    - 950 µL per well of a MACSwell Two or MACSwell One Small
    - 475 µL per well of a MACSwell Four Imaging Frame
- Add the appropriate initial sample volume of MACSima Running Buffer:
    - 1900 µL per well of a MACSwell One
    - 950 µL per well of a MACSwell Two or MACSwell One Small
    - 475 µL per well of a MACSwell Four Imaging Frame

Note: Short term storage (max. 1 week) of the slide in the MACSwell Imaging Frame filled with MACSima Running Buffer is possible at 4 °C.

2.2 Acetone fixation

Material:

- Slide with frozen non-fixed tissue section
- -20 °C cold Acetone (Sigma-Aldrich/Merck, # 650501-1L)
- MACSima Running Buffer (Miltenyi Biotec, # 130-121-565)
- Petri dish (glass) (Th Geyer, # 9170451)
- MACSwell One Imaging Frames (# 130-124-673), MACSwell One Small Imaging Frames (Miltenyi Biotec, # 130-126-794), MACSwell Two Imaging Frames (# 130-124-675), MACSwell Four Imaging Frames (# 130-124-676)

- Remove the slide with frozen non-fixed tissue from cryostat or dry ice.
- Immerse the slide with frozen non-fixed tissue section in -20°C cold acetone in a petri dish and incubate for 3 minutes.
- Remove the slide and let the residual acetone evaporate shortly
- Store slide at -80°C for at least one day.
- Remove the slide from freezer.

- Immerse the slide in -20°C cold acetone in a petri dish and incubate for 10 minutes
- Air dry the slide for 30 minutes at room temperature.
- Proceed immediately with mounting the selected MACSwell Imaging Frame (as described in the MACSwell Imaging Frames datasheet) on the slide and add the appropriate initial sample volume of MACSima Running Buffer:
    - 1900 µL per well of a MACSwell One
    - 950 µL per well of a MACSwell Two or MACSwell One Small
    - 475 µL per well of a MACSwell Four Imaging Frame

Note: Directly proceed with further processing and do not store the acetone-fixed samples to avoid changes in tissue morphology.

3. Pre-staining of nuclei using DAPI

Material:

- MACSwell Imaging frame(s) containing fixed sections
- DAPI Staining Solution (Miltenyi Biotec, component of MACSima Stain Support Kit: # 130-127-574 (human), # 130-127-575 (mouse))
- MACSima Running Buffer (Miltenyi Biotec, # 130-121-565)

- Right before the start of the MICS experiment a DAPI pre-staining should be performed.
- Prepare the DAPI pre-staining solution by diluting DAPI Staining Solution at 1:5 (1 µg/ml) for tissue sections in MACSima Running Buffer at final volumes of
    - 1000 µL per well of a MACSwell One
    - 500 µL per well of a MACSwell Two or MACSwell One Small
    - 250 µL per well of a MACSwell Four Imaging Frame

  Note: The optimal dilution factors of DAPI Staining Solution may vary depending on sample type and might need to be adapted.
- Remove the MACSima Running Buffer from the sample(s) by pipetting
- Add the DAPI pre-staining solution and incubate for 10 minutes in the dark at room temperature

- Afterwards, remove the DAPI pre-staining solution and wash the slide three times with MACSima Running Buffer depending on the used MACSwell Imaging Frame:
    - 1900 µL per well of a MACSwell One
    - 950 µL per well of a MACSwell Two or MACSwell One Small
    - 475 µL per well of a MACSwell Four Imaging Frame
- Add MACSima Running Buffer to each well depending on the used MACSwell Imaging Frame:
    - 1900 µL per well of a MACSwell One
    - 950 µL per well of a MACSwell Two or MACSwell One Small
    - 475 µL per well of a MACSwell Four Imaging Frame

  Note: Make sure that the initial sample volume is correct before loading the sample into the MACSima Imaging System.
- Follow the instructions of the MACSima Imaging System User Manual & the instrument software to start the experiment.