MACSima™ Imaging Cyclic Staining (MICS)
Sample preparation protocol for FFPE 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 microtome

Material:

- Formalin fixed and paraffin-embedded tissue sample. 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)
- Heat plate (e.g. Kunz Instruments, # HPL-2)
- Microtome (e.g. Leica, Microm HM 355S)
- Incubator (40 °C)

- Cut 3 µm thick sections using the microtome
- Mount the required number of sections (depending on MACSwell format) onto a slide. Use the positioning template indicating the optimal position of the sections on the slide.
- For flattening & adhering of sections onto the slides, incubate the slide(s) with the sections on the heat plate for 45 min at 40 °C
- Incubate the slide(s) with the sections overnight in an incubator at 40 °C.
- Slides can be stored at room temperature. Storage at room temperature over prolonged times can affect the immunostaining performance. If possible process prepared slides within 1 week.

2. Deparaffinization and antigen retrieval

Heat-induced epitope retrieval (HIER) at basic conditions (pH 9) according to this protocol is recommended for our catalog of MICS-validated antibodies as all of our FFPE-validated antibodies have been tested using this protocol. Depending on combinations of antibodies and tissues the optimal antigen retrieval conditions might vary from the standard and might need to be optimized empirically.

Material:

- Slides containing FFPE-fixed tissue sections
- Epredia Lab Vision PT-Module (Thermo Fisher, # A80400012)
- Staining troughs (e.g. Carl Roth, # H550.1)
- Xylene (e.g. Carl Roth, # 9713.3)
- Ethanol 100 %, 95 %, 80 %, 70 %, 50 % (e.g. Sigma-Aldrich/Merck, # 100983)
- 10 M NaOH solution (to be prepared from e.g. Sigma-Aldrich/Merck, # V000101)
- TEC Buffer pH 9 (for preparation see below)
- MACSima Running Buffer (Miltenyi Biotec, # 130-121-565)

TEC buffer preparation:

TEC Buffer pH 9, 10x stock solution (1 l)

2.5 g Trizma Base (Sigma-Aldrich/Merck, # 93362)
5.75g EDTA Disodium salt dihydrate (Sigma-Aldrich/Merck, # E5134)
3.2 g Sodium citrate tribasic dihydrate (Merck, # 1064480500)

- Solve the chemicals in a final volume of 1 l dH_2O (distilled water).
- Use 10 M NaOH to set the pH to 9.
- To obtain the 1x TEC buffer, dilute the 10x stock solution with dH_2O 1:10 and verify pH.
- The 10x stock solution can be stored at 4°C for up to 3 months
- Prepare 1x solution freshly before use

Deparaffinization

- Transfer the slides in the following solutions. Take care that there are no remaining streaks on the slides after transferring them into the buffer, if so lift the slide up and put them down in the buffer over and over until streaks have disappeared.
    - Immerse the slides in xylene for 20 min
    - Immerse the slides in 100 % EtOH for 1 min
    - Immerse the slides in 95 % EtOH for 1 min
    - Immerse the slides in 80 % EtOH for 1 min
    - Immerse the slides in 70 % EtOH for 1 min
    - Immerse the slides in 50 % EtOH for 1 min
    - Immerse the slides in dH_2O for 1 min

Antigen retrieval

The following steps are performed using the PT-Module:

- Preheat TEC buffer pH 9 to 85 °C
- Place slides in the buffer
- Allow it to warm up to 98 °C
- Incubate at 98 °C for 20 minutes
- Allow it to cool to 85 °C
- Remove slides and proceed immediately with the next step, the tissue sections must not dry out.

3. Mounting of MACSwell Imaging Frame(s) onto slides

Material:

- MACSima Running Buffer (Miltenyi Biotec, # 130-121-565)
- 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)

- Proceed immediately with mounting the selected MACSwell Imaging Frame on the slide (as described in the MACSwell Imaging Frames datasheet) 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: Short term storage (max. 1 week) of the mounted slide filled with MACSima Running Buffer is possible at 4 °C .

4. 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 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 Frames

  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.