Miltenyi Biotec

MACSima™ Stain Support Kit
human

Order no. 130-127-574

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1. Description

This product is for research use only.

Components **1×1 mL DAPI Staining Solution**
1×1 mL FcR Blocking Reagent, human
2×1 mL REAlease® Release Reagent
2× MACSima Mixing Vials (empty)
5× MACSima Septum Caps

NOTICE **MACSima Septum Caps are only compatible with MACSima Systems running MBCore operating system.** Using MACSima Systems running Windows® operating system can damage the robotic needle arm.

- Ensure to use MACSima System running MBCore operating system.

Product format DAPI Staining Solution is supplied in distilled water at a concentration of 5 µg/mL. FcR Blocking Reagent and REAlease Release Reagent are supplied in buffer containing 0.05% sodium azide and stabilizer.

Storage Store protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the vial labels. Do not use after this date.

1.1 Background information

The MACSima Stain Support Kit is designed for use with the MACSima System. The MACSima System is a fully automated instrument capable of staining hundreds of markers on one sample using the MACSima Imaging Cyclic Staining (MICS) technology. The kit contains support reagents and mixing vials necessary to perform experiments with the MACSima System. DAPI Staining Solution is used to stain cell nuclei. FcR Blocking Reagent is used to block unwanted binding of antibodies to human Fc receptor-expressing cells such as B cells, monocytes, and macrophages. REAlease Release Reagent is used for signal removal from samples stained with REAlease or REAdye_lease™ Antibody Complexes.

1.2 Reagent and instrument requirements

- MACSima System (# 130-121-164) running MBCore operating system.
- MACSima Running Buffer (# 130-121-565)
- MACSwell One Imaging Frames (# 130-124-673), MACSwell Two Imaging Frames (# 130-124-675), MACSwell Four Imaging Frames (# 130-124-676), or MACSwell 24 Imaging Plates (# 130-124-677)
- (Optional) Dissolved fluorochrome-conjugated antibodies. For more information about MICS–pre-tested antibodies refer to www.miltenyibiotec.com.
- (Optional) MACSwell Deepwell Plates (# 130-126-865)
- (Optional) MACSwell Sealing Foils (# 130-126-866)
- (Optional) REAscreen Antibody Panels containing dried antibodies

2. Use of the MACSima Stain Support Kit with dissolved antibodies and MACSwell Deepwell Plates

▲ When using the MACSima Septum Caps to prevent drying of the reagents in a long experiment, the MACSima System must be equipped with MBCore operating system.

▲ Plan the experiment beforehand in the MACSima Software.

▲ Upon experiment planning, the MACSima Software will indicate the number of MACSima Stain Support Kits and further material needed. It will also indicate the volumes needed of each reagent for preparation of the final staining solutions in the individual wells of the MACSwell Deepwell Plates.

2.1 Preparation of the MACSwell Deepwell Plates

▲ For detailed information on MACSwell Deepwell Plates please refer to the respective data sheet.

1. Add MACSima Running Buffer into the MACSwell Deepwell Plate.

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2. Add DAPI Staining Solution into the dedicated well positions on the MACSwell Deepwell Plate.
3. (Optional) Add FcR Blocking Reagent into the well positions on the MACSwell Deepwell Plate.

   ▲ **Note:** When using REAfinity®/REAlease/REAdye_lease Antibodies only, it is not required to add FcR Blocking Reagent.
4. Add the dissolved fluorochrome-conjugated antibodies at their respective final dilution into their dedicated well positions on the MACSwell Deepwell Plate and resuspend by manually pipetting up and down.
5. Seal the MACSwell Deepwell Plate with a MACSwell Sealing Foil. For this, carefully detach the protection film of the adhesive side holding the foil at the protected margin. Accurately place the foil above the plate and attach it to the surface of the plate. Make sure all wells are covered.

   ▲ **Note:** MACSwell Deepwell Plates can only be sealed with MACSwell Sealing Foils due to requirements of the MACSima System.

2.2 Preparation of samples

1. Prepare sample(s) for the experiment as described in the respective MACSwell Imaging Frames data sheet and add the initial sample volume of MACSima Running Buffer to each well depending on the used MACSwell Imaging Frame:

   1900 µL for MACSwell One,

   950 µL for MACSwell Two, or

   475 µL for MACSwell Four Imaging Frames or MACSwell 24 Imaging Plates.

   ▲ **Note:** Make sure that the initial sample volume is correct before loading the sample into the MACSima System.
2. In case of manual pre-staining of cell nuclei with DAPI prior to the MICS experiment, proceed with steps 3–6. If no manual pre-staining is performed, directly proceed with chapter 2.3.
3. (Optional) Prepare the pre-staining solution by diluting DAPI Staining Solution 1:5 for tissue sections and adherent cells, or 1:25 for suspension cells in MACSima Running Buffer with a final volume per well depending on the used MACSwell Imaging Frame:

   1000 µL for MACSwell One,

   500 µL for MACSwell Two, or

   250 µL for MACSwell Four Imaging Frames or MACSwell 24 Imaging Plates.

   ▲ **Note:** The optimal dilution factor of DAPI Staining Solution may vary depending on the sample type and might need to be adjusted.
4. (Optional) 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 (+19 to +25 °C).
5. (Optional) Remove the pre-staining solution and wash the sample(s) three times by adding MACSima Running Buffer to each well depending on the used MACSwell Imaging Frame:

   1900 µL for MACSwell One,

   950 µL for MACSwell Two, or

   475 µL for MACSwell Four Imaging Frames.
6. (Optional) Add MACSima Running Buffer to each well depending on the used MACSwell Imaging Frame:

   1900 µL using MACSwell One,

   950 µL using MACSwell Two, or

   475 µL using MACSwell Four Imaging Frames or MACSwell 24 Imaging Plates.

   ▲ **Note:** Make sure that the initial sample volume is correct before loading the sample into the MACSima System.

2.3 Loading the MACSima System

▲ Please refer to the MACSima System user manual for detailed information on how to use the instrument.

1. Start the MACSima System and software.
2. Refer to the user manual and follow the instructions of the MACSima Software.
3. Load the respective experiment configuration.
4. Prepare only vials which are required by the experiment (REAlease Release Reagent, and, when using, DAPI Staining Solution and/or FcR Blocking Reagent) as indicated by the instrument software. Remove lids from the vials and replace them by screwing MACSima Septum Caps onto the vials.

   ▲ **Note:** Only use MACSima Septum Caps if MACSima System runs with MBCore operating system. Do not use MACSima Septum Caps if system runs with Windows operating system.
5. Remove lids of the empty MACSima Mixing Vials.
6. Load the MACSwell Deepwell Plate sealed with a MACSwell Sealing Foil, the components of the MACSima Stain Support Kit, and the MACSwell Imaging Frame(s) or Plate containing the prepared sample(s) into the instrument as instructed by the MACSima Software.

   ▲ **Note:** Be sure to load the plates in the correct orientation.

   ▲ **Note:** Ensure that MACSima Septum Caps properly screwed onto the vials. The MACSima Mixing Vials must be open, without a septum cap, and empty before placing them into the instrument.

   ▲ **Note:** Ensure that the vials are properly inserted in their clamp holders.
7. Start the experiment by following the instructions of the MACSima Software.
8. After the experiment has finished, follow the instructions of the MACSima Software. Discard the MACSwell Deepwell Plate and the reagent vials.

3. Use of the MACSima Stain Support Kit with dried antibodies on REAscreen Antibody Panels

▲ When using the MACSima Septum Caps to prevent drying of the reagents in a long experiment, the MACSima System must be equipped with MBCore operating system.

▲ Plan the experiment beforehand in the MACSima Software.

▲ Upon experiment planning, the MACSima Software will indicate the material needed.

3.1 Preparation of samples

1. Prepare sample(s) for the experiment as described in the respective MACSwell Imaging Frames data sheet and add the initial sample volume of MACSima Running Buffer to each well depending on the used MACSwell Imaging Frame:

1900 µL for MACSwell One,

950 µL for MACSwell Two, or

475 µL for MACSwell Four Imaging Frames or MACSwell 24 Imaging Plates.

▲ **Note:** Make sure that the initial sample volume is correct before loading the sample into the MACSima System.

2. In case of manual pre-staining of cell nuclei with DAPI prior to the experiment, proceed with steps 3–6. If no manual pre-staining is performed, directly proceed with chapter 3.2.
3. (Optional) Prepare the pre-staining solution by diluting DAPI Staining Solution 1:5 for tissue sections and adherent cells, or 1:25 for suspension cells in MACSima Running Buffer with a final volume per well depending on the used MACSwell Imaging Frame:

   1000 µL for MACSwell One,

   500 µL for MACSwell Two, or

   250 µL for MACSwell Four Imaging Frames or MACSwell 24 Imaging Plates.

   ▲ **Note:** The optimal dilution factor of DAPI Staining Solution may vary depending on the sample type and might need to be adjusted.
4. (Optional) 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 (+19 to +25 °C).
5. (Optional) Remove the pre-staining solution and wash the sample(s) three times by adding MACSima Running Buffer to each well depending on the used MACSwell Imaging Frame:

   1900 µL for MACSwell One,

   950 µL for MACSwell Two, or

   475 µL for MACSwell Four Imaging Frames.
6. (Optional) Add MACSima Running Buffer to each well depending on the used MACSwell Imaging Frame:

   1900 µL using MACSwell One,

   950 µL using MACSwell Two, or

   475 µL using MACSwell Four Imaging Frames or MACSwell 24 Imaging Plates.

   ▲ **Note:** Make sure that the initial sample volume is correct before loading the sample into the MACSima System.

3.2 Loading the MACSima System

▲ Please refer to the MACSima System user manual for detailed information on how to use the instrument.

1. Start the MACSima System and software.
2. Refer to the user manual and follow the instructions of the MACSima Software.
3. Load the respective experiment configuration.
4. Unpack the REAscreen Antibody Panel Plate(s) from the pouch.

   ▲ **Note:** Do not remove the sealing or add buffer to the REAscreen Antibody Panel Plate(s). The MACSima System will resuspend the antibodies automatically.
5. Remove lids from the vials containing REAlease Release Reagent, DAPI Staining Solution, and FcR Blocking Reagent. Replace them by screwing MACSima Septum Caps onto the vials. Remove lids of the empty MACSima Mixing Vials.

   ▲ **Note:** Only use MACSima Septum Caps if MACSima System runs with MBCore operating system. Do not use MACSima Septum Caps if system runs with Windows operating system.
6. Load the REAscreen Antibody Panel Plate(s), the components of the MACSima Stain Support Kit, and the MACSwell Imaging Frame(s) or Plate containing the prepared sample(s) into the instrument as instructed by the MACSima Software.

   ▲ **Note:** Be sure to load the plates in the correct orientation.

   ▲ **Note:** Ensure that MACSima Septum Caps properly screwed onto the vials. The MACSima Mixing Vials must be open, without a septum cap, and empty before placing them into the instrument.

   ▲ **Note:** Ensure that the vials are properly inserted in their clamp holders.
7. Start the experiment by following the instructions of the MACSima Software.
8. After the experiment has finished, follow the instructions of the MACSima Software. Discard the REAscreen Antibody Panel Plate(s) and the reagent vials.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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