Miltenyi Biotec

REAscreen™ MAX Kit

human, PFA, version 02

Order no. 130-129-737

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Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components **1× REAscreen MAX, human, PFA, version 02**
3× 96-well round bottom plates containing 205 dried fluorochrome-conjugated antibodies, anti-human

1× MACSima Stain Support Kit, human

Product format REAscreen MAX, human, PFA, version 02 contains dried antibodies containing 0.05% sodium azide. The product format of the MACSima Stain Support Kit, human is listed in the respective data sheet.

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

1.1 Background information

REAscreen MAX Antibody Panels are pre-defined and ready-to-use 96-well plates containing dried fluorochrome-conjugated antibodies for use in the MACSima Imaging System. The MACSima Imaging System is a fully automated instrument capable of staining hundreds of markers on a single sample using MACSima Imaging Cyclic Staining (MICS) technology. The REAscreen MAX, human, PFA, version 02 Antibody Panel contains 205 MICS–pre-tested fluorochrome-conjugated antibodies to detect 205 biomarkers on human paraformaldehyde (PFA)-fixed samples using Miltenyi Biotec's antibody portfolio. Each REAscreen MAX Kit includes a MACSima Stain Support Kit which contains support reagents required for a MICS experiment.

Refer to **www.miltenyibiotec.com/130-129-737** for information on the panel composition.

1.2 Applications

Ultra-high content imaging analysis using the MACSima Imaging System for the identification of marker expression for, e.g.,:

- Deep phenotyping, e.g., of the tumor immune microenvironment.
- Drug target discovery and validation.
- Biomarker research and discovery.

One REAscreen MAX Antibody Panel provides antibodies for staining of either one sample in a MACSwell Two Imaging Frame, two samples in a MACSwell Four Imaging Frame, or two samples in a MACSwell 24 Imaging Plate.

1.3 Reagent and instrument requirements

- MACSima Imaging System (# 130-121-164)
- MACSima Running Buffer (# 130-121-565)
- Standard microscope slide (75–76 mm × 25–26 mm × 1 mm) or one variant of MACSwell Micro Slide, e.g., MACSwell Micro Slide (17 µm) (# 130-124-678)
- MACSwell™ Two Imaging Frames (# 130-124-675), MACSwell Four Imaging Frames (# 130-124-676), or MACSwell 24 Imaging Plates (# 130-124-677)

	Number of wells/samples
MACSwell Two	1
MACSwell Four	2
MACSwell 24	2

Table 1: Supported well or sample number per REAscreen MAX Antibody Panel.

2. Protocol

▲ Plan the experiment beforehand in the MACSima Software.

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

2.1 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:

   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 Imaging 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 2.2.

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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:

   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 (19–25 °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:

   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:

   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 Imaging System.

2.2 Loading the MACSima Imaging System

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

1. Start the MACSima Imaging 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 Imaging System will resuspend the antibodies automatically.

5. 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 plate in the correct orientation.

   ▲ **Note:** Remove the lids of the reagent vials and ensure that the MACSima Mixing Vials are open and empty before placing them into the instrument.

6. Start the experiment by following the instructions of the MACSima Software.

   ▲ **Note:** Check fill status of the system buffer daily. If it is running low, replace it with a new bottle during the imaging step.

   ▲ **Note:** Check fill status of the waste bottle daily. Empty it if needed to avoid run pauses caused by full waste bottle.

7. 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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