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## 1. Description

This product is for research use only.

<b>Components</b>	4× MACS Marker Screen Plates, anti-human, version 02 (96-well round bottom plates) containing 382 pre-titrated anti-human antibodies, including 6 isotype controls, conjugated to APC. <b>8× adhesive foils</b>
<b>Capacity</b>	One assay with up to $2.5 \times 10^5$ total cells per well.
<b>Product format</b>	Dried antibodies containing 0.05% sodium azide.
<b>Storage</b>	Store MACS Marker Screen Plates protected from light at +2 to +8°C. Do not freeze. Store foils at room temperature. The expiration date of the sealed plates is indicated on the plate label. For information about reconstitution of the dried antibodies and storage after reconstitution refer to chapter 2.2.

### 1.1 Background information

MACS Marker Screen, anti-human, version 02 has been designed for screening and analysis of 376 human surface markers by flow cytometry. It combines the benefits of flow cytometry and high quality pre-titrated antibodies to screen for markers of interest in a standardized and high-throughput manner. Identified markers or combinations of markers can serve to better characterize cell compositions or cells of interests, e.g., for quality control purposes. Furthermore, markers can be utilized to develop target cell isolation strategies.

Refer to [www.miltenyibiotec.com/130-127-043](http://www.miltenyibiotec.com/130-127-043) for information on the plate map, list of antibodies, and performance data.

## 1.2 Applications

Identification of surface marker expression for, e.g.,:

- Characterization or profiling of cell lines or primary cells and cell mixtures.
- Identification of expression changes with different experimental conditions, e.g., variation of cultivation media, upon cytokine stimulation, co-cultivation, differentiation, etc.
- Development of cell separation strategies.

## 1.3 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS Rinsing Solution (# 130-091-222). Keep buffer cold (+2 to +8 °C).

▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  are not recommended for use.

- Flow cytometer equipped with a red (640 nm) laser, e.g., MACSQuant® Analyzer 10 (# 130-096-343) or MACSQuant Analyzer 16 (# 130-109-803) or other flow cytometer able to run 96-well plates.
- Adjustable multichannel pipettes for measuring volumes ranging from 25–200 µL and respective reservoirs.
- Centrifuge with a rotor and adapter for 96-well plates.
- Deionized water.
- (Optional) Transcription Factor Staining Buffer Set (# 130-122-981) for staining of transcription factors.
- (Optional) Inside Stain Kit (# 130-090-477) for staining of cytosolic proteins for, e.g., cytokines or structural proteins.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells in unfixed samples.
- ▲ **Note:** Do not use when working with fixed cells or in combination with the Transcription Factor Staining Buffer Set or the Inside Stain Kit.
- (Optional) Viability™ Fixable Dyes, e.g., Viability 405/452 Fixable Dye (# 130-130-421) for flow cytometric exclusion of dead cells in fixed samples.
- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693), anti-mouse Igκ (# 130-097-900), or anti-human Igκ (# 130-104-187) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

## 2. Protocol

### 2.1 Sample preparation

▲ Always prepare cells before reconstitution of antibodies.

▲ For surface staining it is recommended to use  $1\text{--}2.5 \times 10^5$  cells per well. Prepare 10.5 mL of cell suspension at a concentration of  $0.4\text{--}1 \times 10^7$  cells per mL.

▲ It is recommended to perform staining of intracellular proteins after cell surface staining. For intracellular staining, use  $2.5 \times 10^5$  cells per well. This corresponds to  $1 \times 10^7$  cells/mL.

1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in buffer for a concentration of  $0.4\text{--}1 \times 10^7$  cells/mL.
4. Store cell suspension in the dark in the refrigerator (+2 to +8 °C) until use.

### 2.2 Reconstitution of antibodies

1. Centrifuge MACS Marker Screen Plates at  $300 \times g$  for 5 minutes.
2. Prepare 10.5 mL reconstitution solution by mixing Buffer and deionized water 1:1.
3. Carefully unseal the plates.
4. Add 25  $\mu\text{L}$  of reconstitution solution per well using a multichannel pipette.
5. Incubate for 10 minutes in the dark at +2 to +8 °C.
6. Perform a short spin to avoid drops on the edge.
7. Immediately proceed with cell surface staining (refer to chapter 2.3).

### 2.3 Cell surface staining

▲ Prepare stained cells for flow cytometer setup and compensation separately.

▲ Make sure to keep incubation times constant for each plate as pipetting of each plate may take 2–3 minutes.

1. Add 25  $\mu\text{L}$  of cell suspension per well to the MACS Marker Screen Plates prepared before (refer to chapter 2.2). Gently mix the cell suspension by pipetting up and down 2–3 times. Change pipette tips between each row.
2. Incubate for 10–30 minutes in the dark in the refrigerator (+2 to +8 °C).
3. Wash cells by adding 200  $\mu\text{L}$  of buffer per well and centrifuge at  $300 \times g$  for 5 minutes.
4. Quickly invert the plate to discard the supernatant. Gently blot the plate on paper towel for a second to remove the remaining liquid around the wells.
5. (Optional) Repeat steps 3 and 4.
6. (Optional) For additional staining of intracellular proteins choose an appropriate fixation and permeabilization protocol, e.g., by using the Transcription Factor Staining Buffer Set (refer to chapter 2.4) or the Inside Stain Kit (refer to chapter 2.5).
7. To directly proceed with flow cytometric analysis (refer to chapter 2.6), resuspend cell pellet in 60  $\mu\text{L}$  of buffer per well.

### 2.4 (Optional) Intracellular staining using the Transcription Factor Staining Buffer Set

▲ Refer to the data sheet of the Transcription Factor Staining Buffer Set for more information.

▲ For flow cytometric exclusion of dead cells in fixed samples use Viability Fixable Dyes, e.g., Viability 405/452 Fixable Dye. Refer to the respective data sheet for more information.

1. Resuspend cell pellet in 50  $\mu\text{L}$  of cold, freshly prepared Fixation/ Permeabilization Solution per well. Gently mix the cell suspension by pipetting up and down 2–3 times. Change pipette tips between each row.
2. Incubate for 30 minutes in the dark in the refrigerator (+2 to +8 °C).
3. Wash cells by adding 200  $\mu\text{L}$  of buffer per well and centrifuge at  $300 \times g$  for 5 minutes.
4. Quickly invert the plate to discard the supernatant. Gently blot the plate on paper towel for a second to remove the remaining liquid around the wells.
5. Wash cells by adding 200  $\mu\text{L}$  of cold 1 $\times$  Permeabilization Buffer per well and centrifuge at  $300 \times g$  for 5 minutes.
6. Quickly invert the plate to discard the supernatant. Gently blot the plate on paper towel for a second to remove the remaining liquid around the wells.
7. Dilute additional antibodies for intracellular staining in cold 1 $\times$  Permeabilization Buffer (required volume is 50  $\mu\text{L}$  per well).
8. Resuspend cell pellet in 50  $\mu\text{L}$  of pre-diluted antibody per well. Gently mix the cell suspension by pipetting up and down 2–3 times. Change pipette tips between each row.
9. Incubate according to manufacturer's instruction.
10. Wash cells by adding 200  $\mu\text{L}$  of cold 1 $\times$  Permeabilization Buffer per well and centrifuge at  $300 \times g$  for 5 minutes.
11. Quickly invert the plate to discard the supernatant. Gently blot the plate on paper towel for a second to remove the remaining liquid around the wells.
12. (Optional) Repeat steps 10 and 11.
13. To proceed with flow cytometric analysis (refer to chapter 2.6), resuspend cell pellet in 60  $\mu\text{L}$  of buffer per well.

### 2.5 (Optional) Intracellular staining using the Inside Stain Kit

▲ Refer to the data sheet of the Inside Stain Kit for more information.

▲ For flow cytometric exclusion of dead cells in fixed samples use Viability Fixable Dyes, e.g., Viability 405/452 Fixable Dye. Refer to the respective data sheet for more information.

1. Resuspend cell pellet in 50  $\mu\text{L}$  of Inside Fix per well. Gently mix the cell suspension by pipetting up and down 2–3 times. Change pipette tips between each row.
2. Mix well and incubate for 20 minutes at room temperature.
3. Centrifuge at  $300 \times g$  for 5 minutes.
4. Quickly invert the plate to discard the supernatant. Gently blot the plate on paper towel for a second to remove the remaining liquid around the wells.

5. Resuspend pellet in 200  $\mu$ L of buffer.
6. Centrifuge at 300 $\times$ g for 5 minutes.
7. Quickly invert the plate to discard the supernatant. Gently blot the plate on paper towel for a second to remove the remaining liquid around the wells.
8. Dilute additional antibodies for intracellular staining in Inside Perm at appropriate titer according to the manufacturer's instructions. The required volume is 50  $\mu$ L per well.
9. Resuspend cell pellet in 50  $\mu$ L of pre-diluted antibody per well. Gently mix the cell suspension by pipetting up and down 2–3 times. Change pipette tips between each row.
10. Incubate according to manufacturer's instruction.
11. Add 200  $\mu$ L of Inside Perm.
12. Centrifuge at 300 $\times$ g for 5 minutes.
13. Quickly invert the plate to discard the supernatant. Gently blot the plate on paper towel for a second to remove the remaining liquid around the wells.
14. To proceed with flow cytometric analysis (2.6), resuspend cell pellet in 60  $\mu$ L of buffer per well.

## 2.6 Flow cytometric data acquisition with the MACSQuant Analyzer 10 using the analysis template

▲ For more information on how to use the instrument, refer to the user manual and software guide of the flow cytometer in use, e.g., the MACSQuant Analyzer.

▲ Refer to the product page of the MACS Marker Screen, anti-human, version 02 at [www.miltenyibiotec.com/130-127-043](http://www.miltenyibiotec.com/130-127-043) for the analysis template.

1. Prepare and prime the flow cytometer in use, e.g., the MACSQuant Analyzer. Make sure the calibration and instrument settings have been optimized for acquisition of the MACS Marker Screen, anti-human, version 02.
2. (Optional) Import and load the analysis template "MACS<sup>®</sup> Marker Screen, anti-human, version 02".
3. For doublets discrimination choose **Height**. For this, click on the **Advanced button** in the **Channels tab** and click on the **Height button**.
4. (Optional) Add Propidium Iodide Solution or 7-AAD Staining Solution for exclusion of dead cells if the cells were not fixed and permeabilized.
5. Analyze samples using a flow cytometer. It is recommended to analyze at least 10.000 events per well.

▲ **Note:** Refer to the product page of the MACS Marker Screen, anti-human, version 02 at [www.miltenyibiotec.com/130-127-043](http://www.miltenyibiotec.com/130-127-043) for information on performance data.

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