Miltenyi Biotec

BM-MSC Analysis Cocktail Kit, anti-human

Order no. 130-106-646

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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

250 µL BM-MSC Analysis Cocktail, anti-human containing:

CD45 Antibody, anti-human, FITC (clone: 5B1, isotype: mouse IgG2a)

CD235a (Glycophorin A) Antibody, anti-human, VioBlue®, REAfinity™ (clone: REA175, isotype: recombinant human IgG1)

CD271 (LNGFR) Antibody, anti-human, PE (clone: ME20.4-1.H4, isotype: mouse IgG1κ)

250 µL MSCA-1 Antibody, anti-human, APC (clone: W8B2, isotype: mouse IgG1κ)

250 µL BM-MSC Control Cocktail, anti-human containing:

Isotype Control Antibody, mouse IgG1, APC (clone: IS5-21F5, isotype: mouse IgG1κ)

CD45 Antibody, anti-human, FITC (clone: 5B1, isotype: mouse IgG2a)

CD235a (Glycophorin A) Antibody, anti-human, VioBlue, REAfinity (clone: REA175, isotype: recombinant human IgG1)

CD271 (LNGFR) Antibody, anti-human, PE (clone: ME20.4-1.H4, isotype: mouse IgG1κ)

5 mL Red Blood Cell Lysis Solution (10×)

1 mL FcR Blocking Reagent, human

500 µL 7-AAD Staining Solution

Capacity 25 tests with 200 µL human bone marrow sample per test.

Product format Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide. 7-AAD Staining Solution is supplied in phosphate-buffered saline, pH 7.2, at a concentration of 52.5 µg/mL.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1. Background information

The BM-MSC Analysis Cocktail Kit, anti-human has been designed for the quantification of mesenchymal stromal cells (MSCs) in human bone marrow (BM) samples based on the expression of CD271 (LNGFR) and MSCA-1. The kit allows the identification of $CD45^+$ leukocytes and $CD45^{dim}$/CD271 $(LNGFR)^+$/MSCA-1^+ MSCs. CD235a (Glycophorin A) is used to exclude remaining red blood cells, which were not completely lysed by the Red Blood Cell Lysis Solution. The additional MSC marker MSCA-1 allows further the identification of MSCs with highly proliferative potential.
The kit applies recombinantly engineered REAfinity Antibodies. REAfinity Antibodies are recombinant antibodies that provide superior lot-to-lot consistency and purity compared to mouse or rat hybridoma-derived, monoclonal antibodies. They have been recombinantly engineered to produce highly specific antibodies that require no FcR blocking step. Additionally, they all have the same IgG1 isotype, requiring less isotype controls.

1.2. Applications

- Analysis and standardized quantification of human MSCs from fresh bone marrow samples by flow cytometry.

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 (2–8 °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 Ca^{2+} or Mg^{2+} are not recommended for use.

- Flow cytometer equipped with a red (640 nm), a blue (488 nm), and a violet (405 nm) laser, e.g., MACSQuant Analyzer 10 (# 130-096-343) or MACSQuant Analyzer 16 (# 130-109-803).
- Double-distilled water (ddH_2O).
- (Optional) MACSmix™ Tube Rotator (# 130-090-753).
- (Optional) MACS SmartStrainers (100 µm) (# 130-098-463).

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140-004-778.03

- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693) or anti-mouse Igκ (# 130-097-900) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

2. Protocol

▲ For best results use starting material directly after aspiration.

▲ Bone marrow samples have to be supplemented with an anticoagulant, for example, heparin.

▲ Bone marrow samples should be stored at room temperature under agitation until analysis.

▲ When using bone marrow aspirate pass the material through a MACS SmartStrainer (100 µm).

1. Dilute 10× Red Blood Cell Lysis Solution 1:10 with double-distilled water (ddH_2O), for example, dilute 1 mL of 10× Red Blood Cell Lysis Solution with 9 mL of ddH_2O.

   ▲ **Note:** Do not dilute with deionized water. Store prepared 1× Red Blood Cell Lysis Solution at room temperature. Discard unused solution at the end of the day.

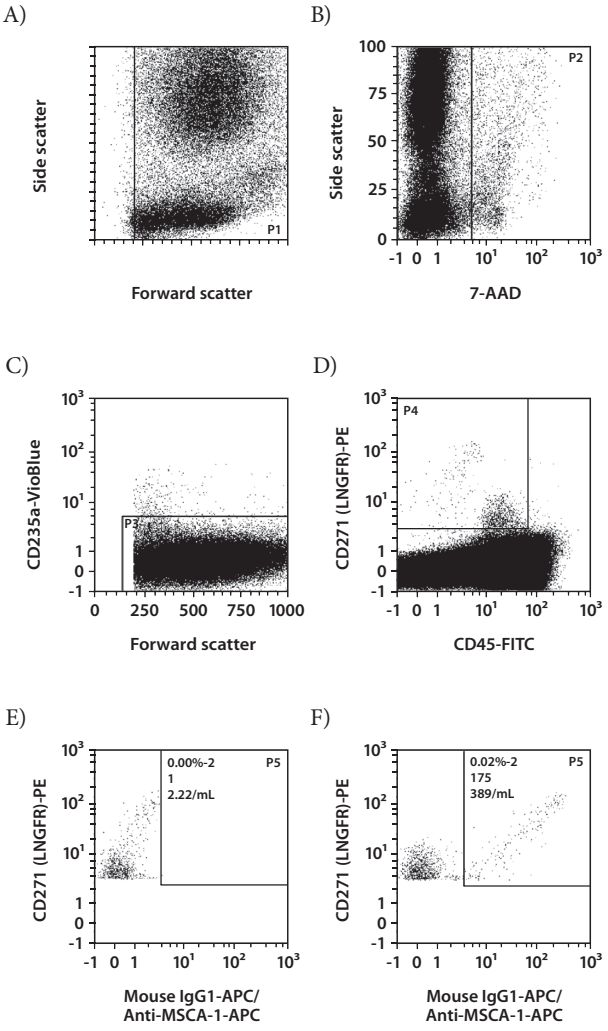
2. Label two tubes with A (control sample) and B (MSC sample).
3. Carefully pipette 100 µL of human bone marrow sample to the bottom of each tube.
4. Add 20 µL FcR Blocking Solution into each tube.
5. Add 10 µL of BM-MSC Control Cocktail, anti-human into tube A and 10 µL of BM-MSC Analysis Cocktail, anti-human as well as 10 µL of MSCA-1 Antibody, anti-human, APC into tube B.
6. Immediately vortex thoroughly and incubate for 10 minutes at 2–8 °C in the dark.
7. Add 860 µL of 1× Red Blood Cell Lysis Solution into tube A and 850 µL in tube B.
8. Immediately vortex and incubate for 10 minutes at room temperature in the dark using the MACSmix Tube Rotator.
9. Add 10 µL of 7-AAD Staining Solution into each tube.
10. Proceed immediately to flow cytometric analysis.

    ▲ **Note:** Analysis should be performed within 20 minutes after staining.

3. Example of immunofluorescent staining with the BM-MSC Analysis Cocktail Kit, anti-human

Bone marrow from a healthy donor was stained with the BM-MSC Analysis Cocktail Kit, anti-human. Staining was carried out at 2–8 °C for 10 minutes. Subsequently, red blood cells were lysed by incubation using 1× Red Blood Cell Lysis Solution at room temperature for 10 minutes. Cells were analyzed by flow cytometry using the MACSQuant Analyzer 10.

A gate around single cells in forward scatter (FSC) versus side scatter (SSC) (A) was set to exclude debris and platelets. To exclude dead cells, a gate around viable cells was set (B). $CD235^+$ erythrocytes were excluded from analysis (C). $CD45^{dim}$/CD271 $(LNGFR)^+$ cells were identified (D). A gate was set within the control sample stained with the BM-MSC Control Cocktail, anti-human (E) to include all $MSCA\text{-}1^+$ events within the MSC sample stained with the BM-MSC Analysis Cocktail, anti-human (F).



3.1. Determination of the MSC number

For the calculation the gate statistic, plot F of the MSC sample has been used. Following region functions are needed:

	Parameter	Description	Example
a	Count/mL	Number of events per mL staining volume	389/mL
b	%–2	Percent grand parent, for information only	0.02%
c	Staining volume	Complete volume after labeling including cocktails, antibody, Red Blood Cell Lysis Solution, FcR Blocking Reagent, and 7-AAD Solution (1000 µL in standard applications)	1000 µL
d	Sample volume	Bone marrow volume used for one staining (100 µL in standard applications)	100 µL

Calculation of the dilution factor

	Parameter	Calculation	Example
e	Dilution factor	c / d	1000 µL/100 µL = 10

Calculation of the MSC count

	Parameter	Calculation	Example
f	Number of MSCs/mL bone marrow	a × e	389/mL × 10 = 3890/mL

In the given example, the count of MSCs is 3890/mL human bone marrow sample.

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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