Miltenyi Biotec

MACSprep™ HLA T Cell Isolation Kit

human

For 5×5 mL whole blood 130-110-128
For 5×20 mL whole blood 130-110-441

Contents

1. Description

This product is for research use only.

Components	**5 vials MACSprep™ HLA T Cell Isolation Cocktail, human – lyophilized**: MACSprep Beads conjugated to monoclonal antibodies. **and** **1×12 mL Buffer A (# 130-110-128)** **1×12 mL Buffer B (# 130-110-128)** or **1×25 mL Buffer A (# 130-110-441)** **1×25 mL Buffer B (# 130-110-441)**
Capacity	For 5×5 mL or 5×20 mL whole blood/spleen cell suspension.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label. For information about reconstitution of the lyophilized cocktail refer to section 2.1.

1.1 Principle of the MACSprep™ Separation

The MACSprep™ HLA T Isolation Kit has been developed for the fast isolation of untouched leukocytes from up to 5×5 mL (# 130-110-128) or 5×20 mL (# 130-110-441) of freshly drawn anticoagulated whole blood or spleen cell suspension without density gradient centrifugation. Erythrocytes are aggregated and sedimented, while non-target cells are removed by immunomagnetic depletion with MACSprep Beads.

The MACSxpress® Separator allows the processing of up to 30 mL whole blood or spleen cell suspension in one isolation step. For cell separation with multiple samples in parallel, the MACSiMAG™ Separator can be used.

1.2 Applications

- Large scale isolation of untouched T cells directly from whole blood without density gradient centrifugation for functional assays or biomarker analysis.
- Compatible with downstream HLA assays (research use only).

1.3 Reagent and instrument requirements

- MACSxpress Separator (# 130-098-308)
- MACSiMAG Separator (# 130-092-168)
- 2 mL, 5 mL, 15 mL, or 50 mL tubes
- MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) MACS SmartStrainers (70 µm) (# 130-098-462, # 130-110-916)
- (Optional) MACSQuant® Analyzer 10 (# 130-096-343)
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45-VioBlue®, CD3-FITC, and CD56-PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

2. Protocol

▲ Use of anticoagulants, e.g., EDTA, heparin, or sodium citrate is recommended.

▲ Adjust all reagents and materials to room temperature (19–25 °C) before use.

▲ Pipette gently to avoid foam formation.

▲ (Optional) For the evaluation of purity and recovery of the target cell fraction, take aliquots where indicated in the protocol.

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2.1 Protocol overview

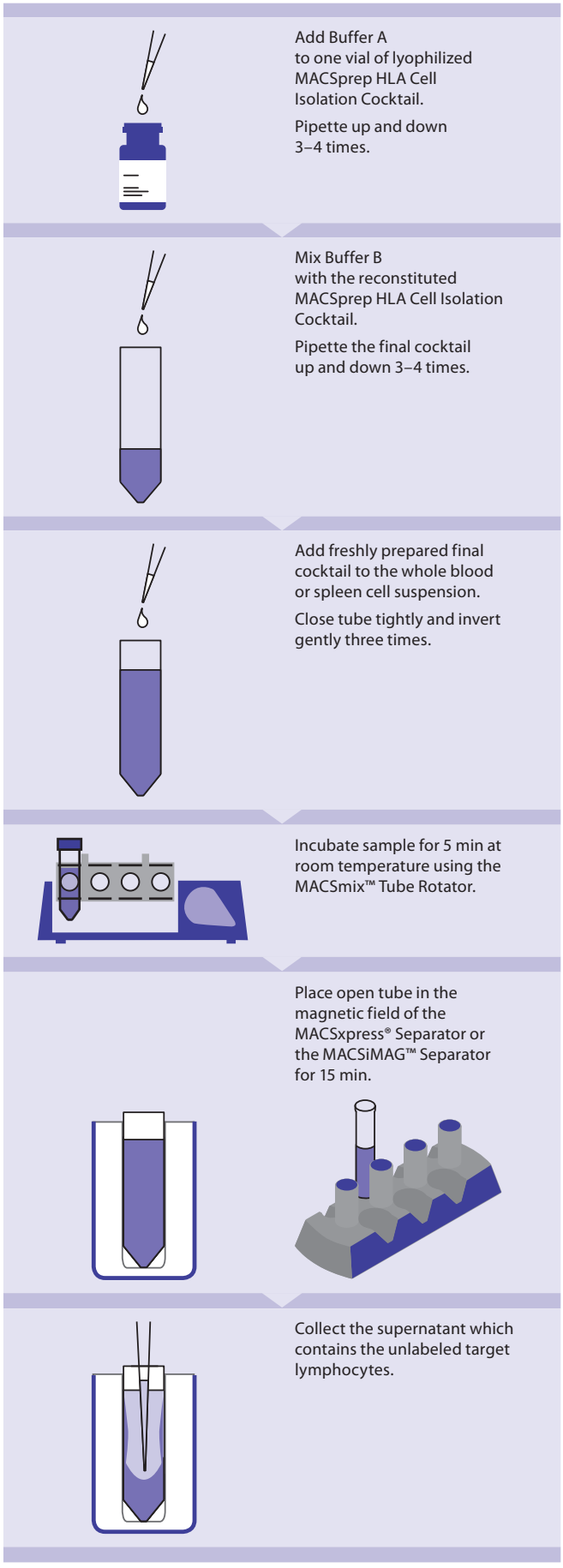

Figure 1: Isolation of leukocytes from whole blood using the MACSprep Cell Isolation Kit.

2.2 (Optional) Preparation of cell suspension from spleen samples

Place spleen sample in a petri dish and perfuse with media (e.g. RPMI) using a syringe several times. Filter cell suspension through a MACS SmartStrainer (70 µm) into a new tube. Determine cell number. It is recommended to use a cell number of 10^5–10^7 cells/mL.

2.3 Preparation of MACSprep™ HLA T Cell Isolation Cocktail

The MACSprep™ HLA T Cell Isolation Cocktail is delivered as a lyophilized pellet. Pellets must be reconstituted immediately before first use.

1. Reconstitute the lyophilized pellet by adding 1.25 mL (# 130-110-128) or 5 mL (# 130-110-441) of Buffer A to one vial of lyophilized MACSprep HLA T Cell Isolation Cocktail. Mix gently by pipetting up and down 3–4 times. This suspension must be homogenous before every use.

   ▲ **Note:** Any unused reconstituted pellet (from step 1) can be stored at 4 °C for up to one week.

2. Prepare the final cocktail by mixing appropriate volumes of the reconstituted pellet from step 1 and Buffer B:

   To process 1 volume of whole blood or spleen cell suspension, 0.25 volumes of the reconstituted pellet (from step 1) and 0.25 volumes of Buffer B are required.

   Example: For 5 mL of whole blood or spleen cell suspension, prepare the final cocktail in a separate tube by adding 1.25 mL of reconstituted pellet to 1.25 mL of Buffer B. Then, mix by gently pipetting up and down 3–4 times. For more examples please see the table 1.

   ▲ **Note:** When working with different volumes of whole blood or spleen cell suspension, adjust reagent volumes accordingly. Pooling of reconstituted pellets might be necessary.

   The final cocktail must be prepared freshly before each cell separation procedure. Use the final cocktail immediately after preparation.

Table 1: Volumes for preparing the appropriate final cocktail.

	Final cocktail to be prepared	
Sample volume	**Volume of reconstituted pellet**	**Volume of Buffer B**
1 mL	0.25 mL	0.25 mL
5 mL	1.25 mL	1.25 mL
10 mL	2.5 mL	2.5 mL
20 mL	5 mL	5 mL
25 mL	6.25 mL	6.25 mL

▲ **Note:** Any unused reconstituted pellet (from step 1) can be stored at 4 °C for up to one week.

The MACSprep™ HLA T Cell Isolation Cocktail is now ready to use. Proceed to magnetic labeling (2.4).

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2.4 Magnetic labeling

▲ Reagent volumes for magnetic labeling given below are for 5 mL of whole blood or spleen cell suspension. When working with different sample volumes, adjust reagent volumes accordingly, e.g., for 10 mL of whole blood or spleen cell suspension use 5 mL of final cocktail. Consult the tables below for the appropriate tube size when using the MACSxpress® Separator or the MACSiMAG™ Separator.

Table 2: Volumes when using the MACSxpress Separator.

Sample volume	Max. final volume (sample plus cocktail)	Recommended tube
up to 2 mL	3 mL	5 mL
2–8 mL	12 mL	15 mL
9–25 mL	37.5 mL	50 mL

Table 3: Volumes when using the MACSiMAG Separator.

Max. sample volume	Max. final volume (sample plus cocktail)	Recommended tube	Max. number of parallel separations
1 mL	1.5 mL	2 mL	8
2 mL	3 mL	5 mL	8
6 mL	9 mL	15 mL	4
16 mL	24 mL	50 mL	3

▲ **Note:** When using the MACSiMAG Separator, refer to the respective data sheet for correct position of the tube rack.

1. (Optional) Take an aliquot of whole blood or spleen cell suspension for cell counting and staining to determine target cell frequency in the starting material (refer to section 2.6).
2. Pipette 5 mL of anticoagulated whole blood or spleen cell suspension into a 15 mL tube.
3. Add 2.5 mL of final cocktail to the whole blood or spleen cell suspension.
4. Close the tube tightly and invert gently three times. Incubate sample for 5 minutes at room temperature using the MACSmix™ Tube Rotator on permanent run speed of approximately 12 rpm.

   ▲ **Note:** If another rotator is used, make sure to adjust rotation speed.
5. Proceed to magnetic separation (2.5).

2.5 Magnetic separation

1. Remove the tube containing the sample from the MACSmix Rotator and carefully open the cap.
2. Place the open tube in the magnetic field of the MACSxpress or MACSiMAG Separator for 15 minutes. The magnetically labeled cells will adhere to the wall of the tube while the aggregated erythrocytes sediment to the bottom.

   ▲ **Note:** Do not move the tube during the separation process.
3. While the tube is still inside the MACSxpress or MACSiMAG Separator, carefully collect the supernatant in a new 15 mL tube. For optimal recoveries, collect supernatant by moving the pipette tip top-to-bottom down the front wall of the tube (fig. 1). The supernatant contains the target cell fraction.

   ▲ **Note:** The MACSiMAG Separator is not equipped with a background light. It is recommended to place a light source on the laboratory bench to distinguish between supernatant and sedimented cells.

   ▲ **Note:** Leave a residual volume of supernatant (approximately 1–2 mm above erythrocyte pellet) to avoid unintended aspiration of erythrocytes. If any erythrocytes are aspirated: keep the tube in the separator and pipet the liquid back into the tube. Incubate for 5 minutes and take off the supernatant.
4. (Optional) Take an aliquot of the supernatant for cell counting and staining after magnetic separation (refer to section 2.6).

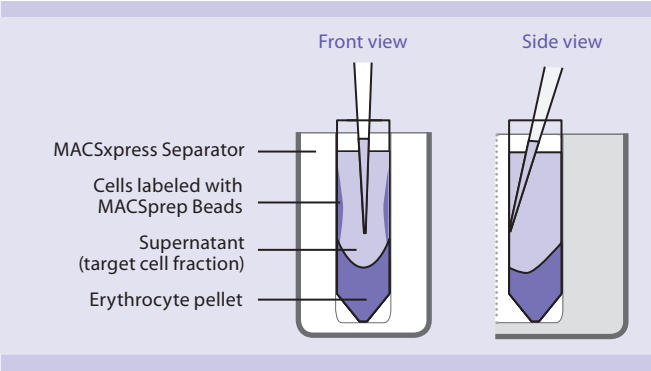


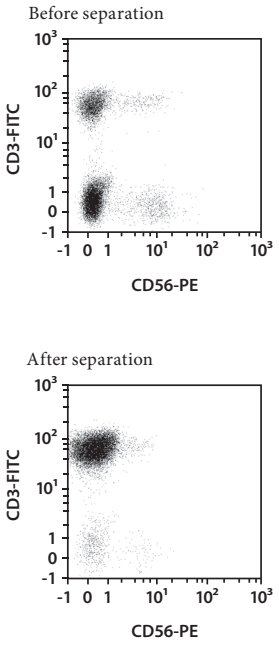
Figure 1: Front and side view of the MACSxpress Separator containing a separated blood sample in a 50 mL tube.

2.6 (Optional) Evaluation of T cell purity

The purity and recovery of the enriched T cells can be evaluated by flow cytometry. Stain an aliquot of each sample fraction collected during the magnetic separation with CD3-FITC, CD56-PE, and CD45-VioBlue® to visualize the target cell fraction. Red blood cells in the fraction before separation should be lysed with Red Blood Cell Lysis Solution (# 130-094-183) prior to flow cytometric analysis. Analyze cells by flow cytometry using the MACSQuant® Analyzer.

3. Example of a separation using the MACSprep™ HLA T Cell Isolation Kit

Untouched CD3+ T cells were isolated from 5 mL of human EDTA-anticoagulated whole blood using the MACSprep™ HLA T Cell Isolation Kit, a MACSmix™ Tube Rotator, and a MACSxpress® Separator. The isolated cells were fluorescently stained with CD45-VioBlue®, CD3-FITC, and CD56-PE and analyzed by flow cytometry using the MACSQuant® Analyzer.
Cell debris, non-leukocytes, and dead cells were excluded from the analysis based on CD45 expression, scatter signals, and propidium iodide fluorescence.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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