Miltenyi Biotec

MACSxpress® LRSC CD4 T Cell Isolation Kit
human

Order no. 130-120-000

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1. Description

This product is for research use only.

Components	**2 vials MACSxpress® LRSC CD4 T Cell Isolation Cocktail, human – lyophilized**: MACSxpress Beads conjugated to monoclonal antibodies.
	1×25 mL MACSxpress Buffer A
	1×25 mL MACSxpress Buffer B
Capacity	For one LRSC.
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 chapter 2.1.

1.1 Principle of the MACSxpress® Separation

MACSxpress® Cell Isolation Kits have been developed for the fast isolation of untouched target cells without density gradient centrifugation. Erythrocytes are aggregated and sedimented, while non-target cells are removed by immunomagnetic depletion with MACSxpress Beads.

1.2 Background information

Leukoreduction system chambers (LRSCs), also known as buffy cones, arise after standard plateletpheresis procedures, where leukocytes are filtered out into the LRSCs. They contain highly concentrated leukocytes in a small volume.

1.3 Applications

- Large scale isolation of untouched CD4$^+$ T cells directly from LRSCs without density gradient centrifugation for functional assays or biomarker analysis.

1.4 Reagent and instrument requirements

- MACSxpress Separator (130-098-308)
- 5 mL polystyrene round-bottom test tube or 15 mL or 50 mL tubes
- (Optional) MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) MACSQuant® Analyzer 10 (# 130-096-343)
- (Optional) MACSxpress Erythrocyte Depletion Reagent (# 130-098-196)
- (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 CD4-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.
- (Optional) 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).

2. Protocol

▲ EDTA as anticoagulant is recommended. Use of other anticoagulants, e.g., heparin or sodium citrate may decrease the yield of target cells.

▲ Bring 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.

2.1 Reagent preparation

▲ If the starting volume of the LRSC is less than 60 mL, dilute by filling up to 60 mL with buffer. Split the sample in two tubes with 30 mL sample volume each.

▲ **Note:** LRSC can be rinsed with buffer to maximize cell recovery from the LRSC. Do not exceed 60 mL sample volume in total.

▲ An isolation mix is made from kit components and must be prepared freshly before each cell separation procedure.

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1. Reconstitute the lyophilized pellet by adding 7.5 mL of Buffer A to one vial of lyophilized MACSxpress LRSC Cell Isolation Cocktail. Mix gently by pipetting up and down 3–4 times. This suspension must be homogenous before every use and can be stored at 4 °C for up to one week.

   ▲ **Note:** Reconstitute one vial of the MACSxpress LRSC Cell Isolation Cocktail per 30 mL diluted LRSC sample.

2. Prepare the isolation mix by mixing appropriate volumes of the reconstituted pellet from step 1 and Buffer B. Use the isolation mix immediately after preparation.

   To process 1 volume of LRSC, 0.25 volumes of the reconstituted pellet (from step 1) and 0.25 volumes of Buffer B are required.

   Example: For 10 mL of diluted LRSC sample, prepare the isolation mix in a separate tube by adding 2.5 mL of reconstituted pellet to 2.5 mL of Buffer B. Then, mix by gently pipetting up and down 3–4 times. For more examples please see the table below.

	Isolation mix to be prepared	
Volume of diluted LRSC to be processed	**Volume of reconstituted pellet**	**Volume of Buffer B**
10 mL	2.5 mL	2.5 mL
30 mL	7.5 mL	7.5 mL

3. Proceed to magnetic labeling (2.2).

2.2 Magnetic labeling

▲ Reagent volumes for magnetic labeling given below are for 30 mL of LRSC sample volume. When working with smaller volumes, scale down reagent volumes accordingly, e.g., use 4 mL of isolation mix for 8 mL of LRSC sample volume, and consult the table below for the appropriate tube size.

LRSC sample volume	Tube size
2–3 mL	5 mL tube
4–8 mL	15 mL tube
9–20 mL	Split sample into several 15 mL tubes
21–30 mL	50 mL tube

1. (Optional) Take an aliquot of LRSC sample for cell counting and staining, to determine target cell frequency in the starting material (refer to section 2.5).
2. Pipette 30 mL of LRSC sample into a 50 mL tube.
3. Add 15 mL of isolation mix to the LRSC sample.
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 it supports overhead mixing of tubes and adjust rotation speed.

5. Proceed to magnetic separation (2.3).

2.3 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® 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 Separator, carefully collect the supernatant in a new 50 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:** Leave a residual volume of supernatant (approximately 1–2 mm above erythrocyte pellet) to avoid unintended aspiration of erythrocytes.

4. (Optional) Take an aliquot of the supernatant for cell counting and staining after magnetic separation (refer to section 2.5).

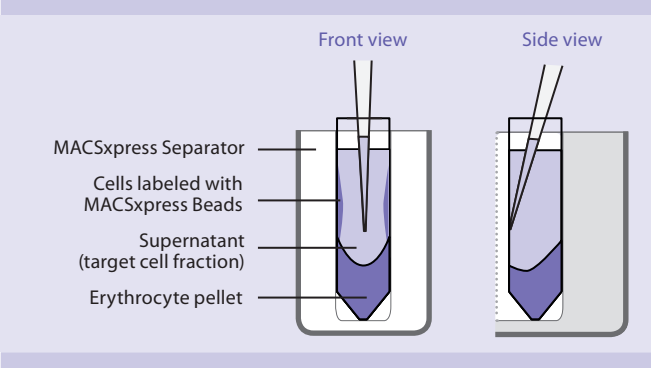


Figure 1: Front and side view of the MACSxpress Separator containing a separated blood sample in a 50 mL tube.

2.4 (Optional) Removal of residual erythrocytes

Residual erythrocytes can be removed by magnetic depletion using the MACSxpress Erythrocyte Depletion Kit (# 130-098-196). Alternatively, erythrocytes can be lysed using the Red Blood Cell Lysis Solution (10×) (# 130-094-183).

Magnetic removal of erythrocytes using the MACSxpress® Erythrocyte Depletion Kit

For removal of erythrocytes using the MACSxpress® Erythrocyte Depletion Kit (# 130-098-196), proceed with the unmodified supernatant from section 2.3, step 3 (i.e. do not centrifuge or dilute). For further instructions please refer to the respective data sheet.

Lysis of erythrocytes using the Red Blood Cell Lysis Solution

1. Centrifuge the supernatant containing the separated $CD4^+$ T cells at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
2. Resuspend the cell pellet with 10 mL of 1× Red Blood Cell Lysis Solution.
3. Proceed according to the Red Blood Cell Lysis Solution data sheet.

▲ (Optional) Take an aliquot of the supernatant for cell counting and staining after erythrocyte lysis (refer to section 2.5).

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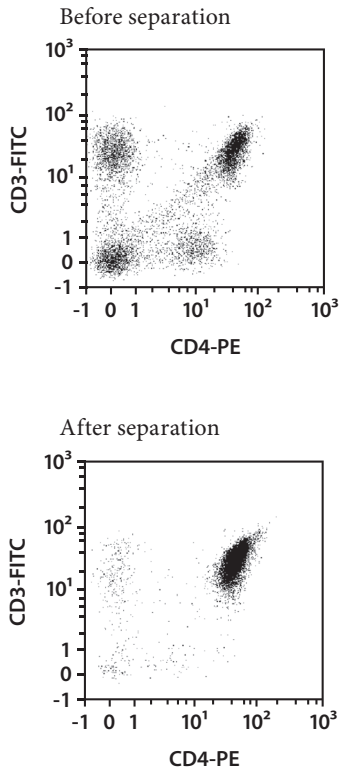
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2.5 (Optional) Evaluation of CD4$^+$ T cell purity

The purity and recovery of the enriched CD4$^+$ T cells can be evaluated by flow cytometry. Stain an aliquot of each sample fraction collected during the magnetic separation with a fluorochrome-conjugated antibody against CD4, e.g., CD3-FITC and CD4-PE. Red blood cells should be lysed or depleted prior to flow cytometric analysis. Analyze cells by flow cytometry using the MACSQuant® Analyzer 10.

3. Example of a separation using the MACSxpress® LRSC CD4 T Cell Isolation Kit

Untouched CD4$^+$ T cells were isolated from 30 mL of LRSC sample using the MACSxpress® LRSC CD4 T Cell Isolation Kit, a MACSmix™ Tube Rotator, and a MACSxpress Separator. The isolated cells were fluorescently stained with CD45-VioBlue®, CD3-FITC, and CD4-PE and analyzed by flow cytometry using the MACSQuant Analyzer 10.
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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