Miltenyi Biotec

MACSxpress® Leukopak® Pan T Cell Isolation Kit
human

Order no. 130-140-326

Contents

1. Description
   - 1.1 Principle of the MACSxpress Separation
   - 1.2 Applications
   - 1.3 Reagent and instrument requirements
2. Protocol
   - 2.1 Reagent preparation
   - 2.2 Magnetic labeling
   - 2.3 Magnetic separation
   - 2.4 (Optional) Evaluation of pan T cell purity
3. Example of a separation using the MACSxpress Leukopak® Pan T Cell Isolation Kit

1. Description

This product is for research use only.

Components	**1 × 2 mL MACSxpress Leukopak® Pan T Cell Isolation Cocktail, human** **4 vials MACSxpress Leukopak® Pan T Cell Isolation Beads, human– lyophilized** **1 × 25 mL MACSxpress Buffer B**
Capacity	For one ½ Leukopak® (5×10^9 total cells, up to 100 mL).
Product format	The MACSxpress Leukopak® Pan T Cell Isolation Cocktail, human is supplied in buffer containing 0.05% sodium azide. The MACSxpress Leukopak® Pan T Cell Isolation Beads, human– lyophilized are supplied in stabilizier.
Storage	Store protected from light at +2 to +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 Applications

- Large scale isolation of untouched pan T cells directly from Leukopak® without density gradient cetrifugation for functional assays or biomarker analysis.

1.3 Reagent and instrument requirements

- MACSxpress Separator (# 130-098-308)

  ▲ **Note:** For 100 mL Leukopak® sample 4 × MACSxpress Separators are needed.
- 5 mL polystyrene round-bottom test tube or 15 mL or 50 mL conical tubes
- Ultra-pure water
- (Optional) MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) MACSQuant® Analyzer 16 (# 130-109-803)
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45 Antibody, anti-human, VioGreen™, REAfinity®, CD3 Antibody, anti-human, VioBlue®, REAfinity, and CD56 Antibody, anti-human, PE, REAfinity. 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 to +8 °C).

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

2. Protocol

▲ The MACSxpress Leukopak® Pan T Cell Isolation Kit has been developed for the negative isolation of target cells from 1/2 Leukopak® with 5 × 10^9 cells (up to 100 mL). Four MACSxpress Separators are needed for simultanous separation of Pan T cells from 100 mL of Leukopak® (25 mL of Leukopak® per MACSxpress Separator).

▲ Bring all reagents and materials to room temperature (+19 to +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

▲ MACSxpress Leukopak® Pan T Cell Isolation Beads must be prepared freshly before cell separation procedure.

1. Reconstitute the lyophilized pellet by adding 1.8 mL of ultra-pure water to one vial of lyophilized MACSxpress Leukopak® Pan T Cell Isolation Beads. 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 four weeks.

   ▲ **Note:** Reconstitute one vial MACSxpress Leukopak® Pan T Cell Isolation Beads per 25 mL Leukopak® sample.

2. Proceed to magnetic labeling (2.2).

2.2 Magnetic labeling

▲ Reagent volumes for magnetic labeling given below are for 25 mL of Leukopak® sample. When working with smaller volumes, scale down reagent volumes accordingly, e.g., use use 2.6 mL MACSxpress Buffer B, 200 µL MACSxpress Leukopak® Pan T Cell Isolation Cocktail, human, and 720 µL MACSxpress Leukopak® Pan T Cell Isolation Beads, human for 10 mL Leukopak® sample, and consult the table below for the appropriate tube size.

Leukopak® sample volume	Tube size
2–3 mL	5 mL tube
4–10 mL	15 mL tube
11–25 mL	50 mL tube

1. (Optional) Take an aliquot of the Leukopak® sample for cell counting and staining, to determine target cell frequency in the starting material (refer to section 2.4).
2. Add 6.25 mL MACSxpress Buffer B and 0.5 mL MACSxpress Leukopak® Pan T Cell Isolation Cocktail into a 50 mL tube.
3. Pipette 25 mL of the Leukopak® sample into the 50 mL tube.
4. Close the tube tightly and invert gently three times. Incubate sample for 10 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. Add 1.8 mL of the reconstituted beads to the Leukopak® sample.
6. 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.

7. 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 (**see Figure 1**). The supernatant contains the target cell fraction.

   ▲ **Note:** Wash the cells before downstream applications.

4. (Optional) Take an aliquot of the supernatant for cell counting and staining after magnetic separation (refer to section 2.4).

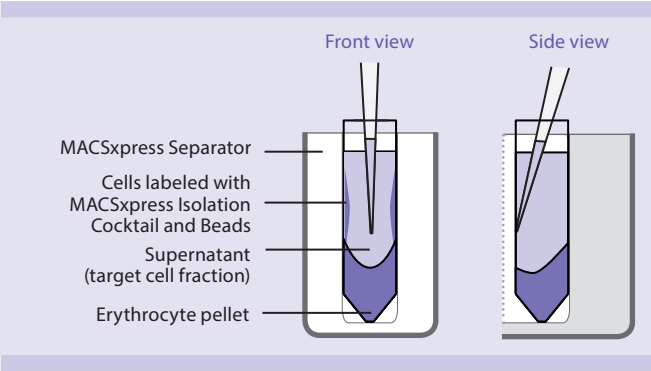


Figure 1: Front and side view of the MACSxpress Separator containing a separated Leukopak® sample in a 50 mL tube.

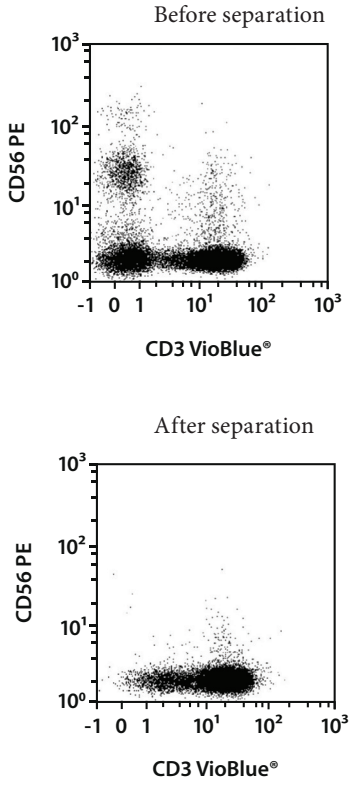
2.4 (Optional) Evaluation of pan T cell purity

The purity and recovery of the enriched pan T cells can be evaluated by flow cytometry. Stain an aliquot of each sample fraction collected during the magnetic separation, e.g., with CD3-VioBlue, and CD56-PE. Red blood cells should be lysed or depleted prior to flow cytometric analysis. Analyze cells by flow cytometry using the MACSQuant Analyzer 16.

3. Example of a separation using the MACSxpress Leukopak® Pan T Cell Isolation Kit

Untouched pan T cells were isolated from 25 mL of Leukopak® sample using the MACSxpress Leukopak® Pan T Cell Isolation Kit, a MACSmix Tube Rotator, and a MACSxpress Separator. The isolated cells were fluorescently stained with CD45-VioGreen, CD3-VioBlue, and CD56-PE analyzed by flow cytometry using the MACSQuant Analyzer 16.

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** for local Miltenyi Biotec Technical Support contact information.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

AutoMACS, MACS, MACSmix, MACSQuant, MACSxpress, the Miltenyi Biotec logo, REAfinity, VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Leukopak is a registered trademark of StemExpress LLC.

Copyright © 2026 Miltenyi Biotec and/or its affiliates. All rights reserved.