Miltenyi Biotec

MACSxpress® Whole Blood NK Cell Isolation Kit
human

Order no. 130-098-185

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) Removal of residual erythrocytes
   - 2.5 (Optional) Evaluation of NK cell purity
3. Example of a separation using the MACSxpress Whole Blood NK Cell Isolation Kit

1. Description

This product is for research use only.

Components	**3 vials MACSxpress® Whole Blood NK Cell Isolation Isolation Cocktail, human – lyophilized**: MACSxpress Beads conjugated to monoclonal antibodies.
	1×25 mL MACSxpress Buffer A
	1×25 mL MACSxpress Buffer B
Capacity	For 3×30 mL whole blood.
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 Applications

- Large scale isolation of untouched NK cells directly from whole blood without density gradient centrifugation for functional assays or biomarker analysis.

1.3 Reagent and instrument requirements

- MACSxpress Separator (# 130-098-308)
- 5 mL polystyrene round-bottom test tube or 15 mL or 50 mL conical tubes
- (Optional) MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) MACSQuant® Analyzer 10 (# 130-096-343)
- (Optional) MACSxpress Erythrocyte Depletion Kit (# 130-098-196)
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45 Antibody, anti-human, VioBlue®, CD3 Antibody, anti-human, FITC, and CD56 Antibody, anti-human, 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

▲ 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

▲ The isolation mix must be prepared freshly before each cell separation procedure.

1. Reconstitute the lyophilized pellet by adding 7.5 mL of MACSxpress Buffer A to one vial of lyophilized MACSxpress Whole Blood 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.
2. Prepare the isolation mix by mixing appropriate volumes of the reconstituted pellet from step 1 and MACSxpress Buffer B. Use the isolation mix immediately after preparation:

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

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

140-003-731.05

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

Volume of whole blood to be processed	Isolation mix to be prepared	
	Volume of reconstituted pellet	Volume of MACSxpress Buffer B
2 mL	0.5 mL	0.5 mL
8 mL	2 mL	2 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 whole blood. When working with smaller volumes, scale down reagent volumes accordingly, e.g., use 4 mL of isolation mix for 8 mL of whole blood, and consult the table below for the appropriate tube size.

Whole blood 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 whole blood for cell counting and staining, to determine target cell frequency in the starting material (refer to section 2.5).
2. Pipette 30 mL of anticoagulated whole blood into a 50 mL tube.
3. Add 15 mL of isolation mix to the whole blood.
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).

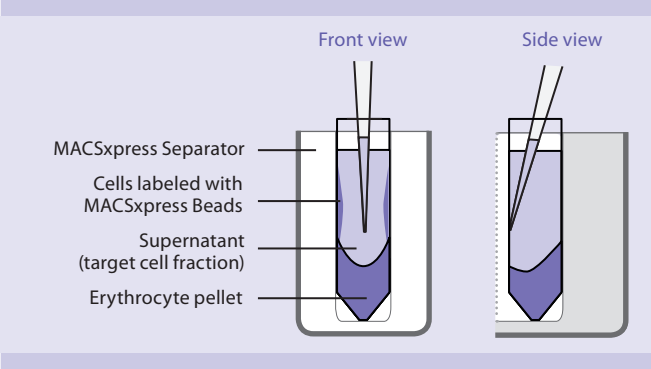


Figure 1: Front and side view of the MACSxpress Separator containing a separated blood sample in a 50 mL tube.

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 step 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 NK 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).

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

140-003-731.05

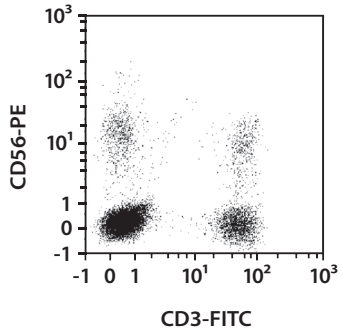
2.5 (Optional) Evaluation of NK cell purity

The purity and recovery of the enriched NK cells can be evaluated by flow cytometry. Stain an aliquot of each sample fraction collected during the magnetic separation, e.g., with CD56 Antibody, anti-human, PE or CD56 Antibody, anti-human, APC and optional, against a T cell marker, e.g., CD3 Antibody, anti-human, FITC. Red blood cells should be lysed or depleted prior to flow cytometric analysis. Analyze cells by flow cytometry using the MACSQuant Analyzer 10.

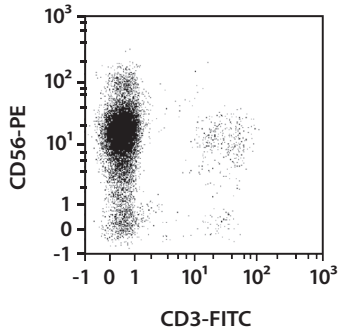
3. Example of a separation using the MACSxpress® Whole Blood NK Cell Isolation Kit

Untouched NK cells were isolated from 30 mL of human EDTA-anticoagulated whole blood using the MACSxpress Whole Blood NK 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 10.
Cell debris, non-leukocytes, and dead cells were excluded from the analysis based on CD45 expression, scatter signals, and propidium iodide fluorescence.

Before separation



After separation



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

MACSmix, MACSQuant, MACSxpress, the Miltenyi Biotec logo, and VioBlue are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.

Copyright © 2021 Miltenyi Biotec and/or its affiliates. All rights reserved.