Miltenyi Biotec

MACSxpress® Buffy Coat NK Cell Isolation Kit
human

Order no. 130-127-696

Contents

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	**3 vials MACSxpress Buffy Coat NK 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 buffy coat (max. 90 mL) from max. 500 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 buffy coat 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, VioGreen™, CD3 Antibody, anti-human, VioBlue®, and CD56-Antibody, anti-human, APC-Vio® 770. 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 buffy coat is less than 90 mL, dilute by filling up to 90 mL with buffer. Split the buffy coat in three tubes with 30 mL sample volume each.

▲ 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 Buffy Coat 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 Buffy Coat Cell Isolation Cocktail per 30 mL buffy coat sample.

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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 buffy coat, 0.25 volumes of the reconstituted pellet (from step 1) and 0.25 volumes of MACSxpress Buffer B are required.

   Example: For 10 mL of diluted buffy coat, 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 diluted buffy coat to be processed	Isolation mix to be prepared	
	Volume of reconstituted pellet	Volume of MACSxpress 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 buffy coat volume. When working with smaller volumes, scale down reagent volumes accordingly, e.g., use 5 mL of isolation mix for 10 mL of buffy coat, and consult the table below for the appropriate tube size.

Buffy coat 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 buffy coat for cell counting and staining, to determine target cell frequency in the starting material (refer to section 2.5).
2. Pipette 30 mL of buffy coat into a 50 mL tube.
3. Add 15 mL of isolation mix to the buffy coat.
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. Centrifuge for 1 minute at 50×g.
6. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

1. Remove the tube containing the sample out of the centrifuge 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).

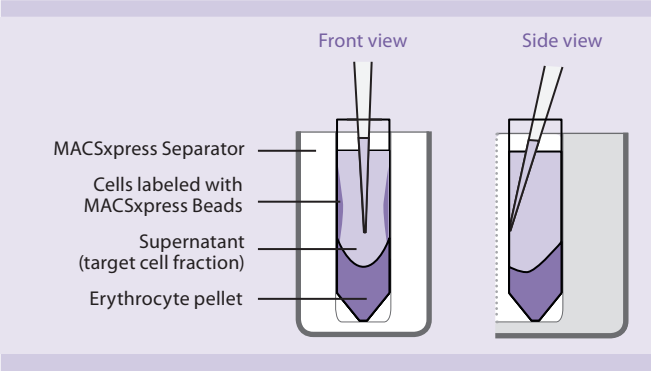

Figure 1: Front and side view of the MACSxpress Separator containing a separated blood sample in a 50 mL tube.

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).

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 CD45 Antibody, anti-human, VioGreen, CD3 Antibody, anti-human, VioBlue, and CD56-Antibody, anti-human, APC-Vio 770. Red blood cells should be lysed or depleted prior to flow cytometric analysis. Analyze cells by flow cytometry using the MACSQuant Analyzer 10.

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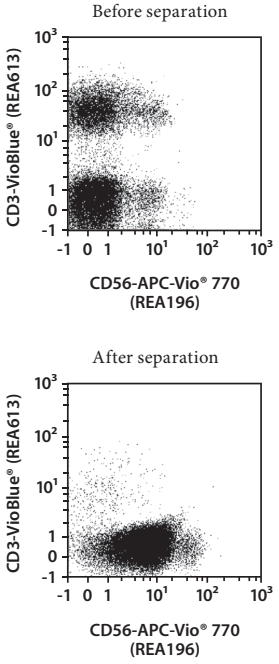
3. Example of a separation using MACSxpress Buffy Coat NK Cell Isolation Kit

Untouched NK cells were isolated from 10 mL of buffy coat using the MACSxpress Buffy Coat NK Cell Isolation Kit, a MACSmix Tube Rotator, and a MACSxpress Separator. The isolated cells were fluorescently stained with Propidium Iodide Solution, CD45-VioGreen, CD3-VioBlue, and CD56-APC-Vio 770 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 for local Miltenyi Biotec Technical Support contact information.

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