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## 1. Description

<b>Components</b>	3 vials MACSxpress® NK Cell Isolation Cocktail, human – lyophilized: MACSxpress Beads conjugated to monoclonal antibodies. 1×25 mL Buffer A 1×25 mL Buffer B
<b>Capacity</b>	For 3×30 mL whole blood.
<b>Storage</b>	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® Isolation Kits have been developed for the fast isolation of untouched leukocytes from up to 30 mL of freshly drawn anticoagulated whole blood 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 tubes
- (Optional) MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) MACSQuant® Analyzer (# 130-092-197)
- (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® (# 130-092-880), CD3-FITC (# 130-080-401), and CD56-PE (# 130-090-755). For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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.
- ▲ 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.

### 2.1 Preparation of MACSxpress® NK Cell Isolation Cocktail

The MACSxpress® NK Cell Isolation Cocktail is delivered as a lyophilized pellet. Pellets must be reconstituted immediately before first use.

1. Reconstitute the lyophilized pellet by adding 7.5 mL of Buffer A to one vial of lyophilized MACSxpress Cell Isolation Cocktail. Mix gently by pipetting up and down 3–4 times. This suspension must be homogenous before every use.
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, 0.25 volumes of the reconstituted pellet (from step 1) and 0.25 volumes of Buffer B are required.

**Example:** For 10 mL of whole blood, prepare the final cocktail 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.

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

Final cocktail to be prepared		
Volume of whole blood to be processed	Volume of reconstituted pellet	Volume of Buffer B
2 mL	0.5 mL	0.5 mL
8 mL	2 mL	2 mL
30 mL	7.5 mL	7.5 mL

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

The MACSxpress NK Cell Isolation Cocktail is now ready to use. 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 final cocktail 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

- (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).
- Pipette 30 mL of anticoagulated whole blood into a 50 mL tube.
- Add 15 mL of final cocktail to the whole blood.
- 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.
 

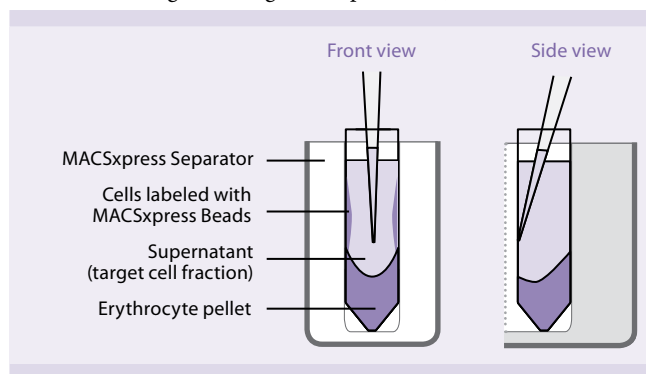
▲ **Note:** If another rotator is used, make sure to adjust rotation speed.
- Proceed to magnetic separation (2.3).

## 2.3 Magnetic separation

- Remove the tube containing the sample from the MACSmix Rotator and carefully open the cap.
- 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.
- 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.
- (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 2.3.3 (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

- Centrifuge the supernatant containing the separated NK cells at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
- Resuspend the cell pellet with 10 mL of 1× Red Blood Cell Lysis Solution.
- Proceed according to the Red Blood Cell Lysis Solution data sheet (step 2.2.2 or 2.3.2).

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

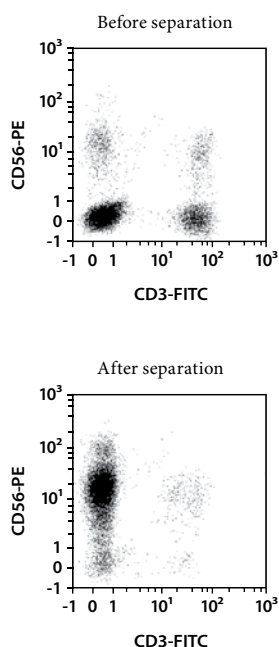
## 2.5 (Optional) Evaluation of NK cell purity

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 with CD56-PE (# 130-090-755) or CD56-APC (# 130-090-843) and optional, against a T cell marker, e.g., CD3-FITC (# 130-080-401). Red blood cells should be lysed or depleted prior to flow cytometric analysis. Analyze cells by flow cytometry using the MACSQuant® Analyzer.

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

Untouched NK cells were isolated from 30 mL of human EDTA-anticoagulated whole blood using the MACSxpress® NK Cell Isolation Kit, a MACSmix Tube Rotator, and a MACSxpress Separator. The isolated cells were fluorescently stained with CD45-VioBlue (# 130-092-880), CD3-FITC (# 130-080-401), and CD56-PE (# 130-090-755) 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.



All protocols and data sheets are available at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

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