Miltenyi Biotec

Multi-24 Whole Blood Column Block

Order no. 130-130-259

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1. Description

This product is for research use only.

Components	**Multi-24 Whole Blood Column Block:** 1× Multi-24 Whole Blood Column Block, 1×24-well Deep Well Plate (sterile packaged)
Storage	Store columns dry at 10–35 °C and protected from light. The expiration date is indicated on the box label. Do not use after this date.

1.1 Principle of the MACS Separation

MACS Technology is based on the use of MACS MicroBeads, MACS Columns, and MACS Separators.
First, the cells are magnetically labeled with the superparamagnetic MACS MicroBeads and the Multi-24 Whole Blood Column Block is placed in the strong permanent magnet of the MultiMACS™ Instrument Family. Then, the labeled blood sample is loaded onto the Multi-24 Whole Blood Column Block. The unlabeled cells run through while the magnetically labeled cells are retained within the Multi-24 Whole Blood Column Block. After transfer of columns to the MACS Elution Station magnetically labeled cells can be eluted from the column.

1.2 Background information

Multi-24 Whole Blood Column Blocks are designed for positive selection of magnetically labeled human whole blood or buffy coat samples from up to 24 samples in parallel with the MultiMACS Cell24 Separator Plus. Multi-24 Whole Blood Column Blocks have been developed for the gentle isolation of cells labeled with StraightFrom® Whole Blood MicroBeads, StraightFrom Buffy Coat MicroBeads, or MACSprep™ Chimerism MicroBeads.
The column matrix of the Multi-24 Whole Blood Column Blocks is composed of ferromagnetic spheres, which are covered with a cell-friendly coating allowing fast and gentle separation of cells. When placed on the MultiMACS Instrument Family, the spheres amplify the magnetic field by 10,000-fold, thus inducing a high gradient within the column.

1.3 Technical specifications

One Multi-24 Whole Blood Column Block is a unit of 24 individual columns, enabling up to 24 separations in parallel. This column block is designed to be used with the MultiMACS Instrument Family.

- Column capacity: up to 10 mL of human whole blood or buffy coat sample per single column.
- Columns are “flow stop” and do not run dry.
- Void volume: 250 µL. Reservoir volume: 6.80 mL.
- Typical flow rate for PBS (phosphate-buffered saline) containing 0.5% BSA (bovine serum albumin): 2.15–2.75 mL/min.
- Multi-24 Whole Blood Column Blocks are for single use only.
- A minimum of 12 columns must be used per run.

1.4 Applications

- Parallel positive selection of cells labeled with StraightFrom Whole Blood MicroBeads or StraightFrom Buffy Coat MicroBeads.

1.5 Reagent and instrument requirements

- 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). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** The recommended buffer is PBS supplemented with EDTA and BSA. The suitability of other buffers has to be tested experimentally.

  ▲ **Note:** Use degassed buffer only! Degas buffer by applying vacuum, preferentially with buffer at room temperature. Excessive gas in running buffer will form bubbles in the matrix during separation. This may lead to clogging of the column and decrease the quality of separation.

- 2×50 mL Whole Blood Column Elution Buffer (# 130-131-889).
- StraightFrom Whole Blood MicroBeads, StraightFrom Buffy Coat MicroBeads, or MACSprep Chimerism MicroBeads for magnetic labeling of cells.
- MultiMACS Cell24 Separator Plus (# 130-098-637) or MultiMACS X (# 130-118-515).
- MultiMACS 5 mL Tube Rack (# 130-095-331) plus 24×5 mL round bottom collection tubes or deep well plates, e.g., Single-well Deep Well Plates (# 130-114-966), 2-well Deep Well Plates (# 130-120-010), or 24-well Deep Well Plates (# 130-110-500).
- (Optional) MACS SmartStrainers (30 µm) (# 130-098-458) to remove cell clumps.

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140-006-077.02

2. Use of Multi-24 Whole Blood Column Blocks

⚠ CAUTION Do not place columns into the MultiMACS Cell24 Separator until the prompt screen **INSERT COLUMNS** appears to ensure that there are no moving parts when placing the columns. This will prevent injuries to fingers that can otherwise become trapped between the MultiMACS Cell24 Magnet and the MultiMACS Column Holder.

▲ In order to be able to use the Multi-24 Whole Blood Column Block, the software version installed on the MultiMACS Cell24 Separator must be equal or higher than the version **4C09**. To verify the current firmware version go to "Set up" -> "Preferences" -> "Show version of Firmware". In case an update is required, please contact the local Miltenyi representative.

▲ The Multi-24 Whole Blood Column Blocks are not suitable for particles larger than 30 µm. To remove clumps and to prevent aggregates in the sample, pass through 30 µm nylon mesh (MACS SmartStrainers (30 µm) # 130-098-458) before separation.

▲ Please use the following buffer volumes for the equilibration, wash, and elution of the Multi-24 Whole Blood Column Block. All buffers are supposed to be used at room temperature.

Equilibration:	1×2 mL separation buffer per single column
Wash:	2×2 mL separation buffer per single column
Elution:	1×4 mL Whole Blood Column Elution Buffer per single column

▲ If only half of the Multi-24 Whole Blood Column Block (12 columns) is used for a magnetic separation, the other half needs to be processed on the same day. Do not store half-used Multi-24 Whole Blood Column Blocks.

2.1 Protocol

1. In the **Start** menu, select either **POSSEL2_24WB** if the cells are collected into a deep well plate or **POSSEL2_24WB5** if the cells are collected into 5 mL tubes. The **Process** menu opens. Tap the **Run** button.
2. **INSERT COLUMNS:** Insert the Multi-24 Column Block into the MultiMACS Column Holder. Tap **OK**.
3. **INSERT WASTE PLATE**: Insert a deep well plate onto the Tip-Touch Plate. Tap **OK**.
4. **APPLY EQUILIBRATION BUFFER:** Apply degassed equilibration buffer onto each column and let the buffer run through. Tap **OK**.
5. **TIP-TOUCH COLUMNS IN PLATE:** Move the Tip-Touch Plate firmly back and forth once. Tap **OK**.
6. **INSERT PLATE TO COLLECT UNLABELED FRACTION:** Remove the waste plate and insert a new deep well plate onto the Tip-Touch Plate. Tap **OK**.

   ▲ **Note:** The MACS Elution Station will generate a vacuum. The status **LED** blinks green or red during this process. When the vacuum has been established, the status **LED** lights up constantly green.
7. **APPLY SAMPLE AND WASH:** Apply the suspension of magnetically labeled cells onto each column and let it run through.
8. Apply degassed wash buffer onto each column and let run through. Ensure that the column reservoir is empty before applying new wash buffer. Tap **OK**.
9. **TIP-TOUCH COLUMNS IN PLATE:** Move the Tip-Touch Plate firmly back and forth once. Tap **OK**. The combined flow-through from the cell suspension and the washes represent the unlabeled target cell fraction.
10. **REMOVE PLATE. PLACE COLUMNS AND PLATE IN ELUTION STATION:** Remove the deep well plate from the Tip-Touch Plate. Insert a new deep well plate into the base of the MACS Elution Chamber to collect the positive fraction from the columns.

    ▲ **Note:** The deep well plate can be inserted in two directions. To ensure the correct assignment of the samples, note the orientation of the deep well plate to the columns.
11. Place either lid I on the base of the MACS Elution Chamber if the cells are collected into a deep well plate or lid II if the cells are collected into 5 mL tubes.
12. Remove the Multi-24 Column Block from the Column Holder and place it on the elution lid (lid I or lid II).
13. Tap **OK** to apply the elution impulse.
14. Wait until the vacuum charge is complete again (see screen instruction).
15. **APPLY ELUTION BUFFER THEN PRESS "OK" IMMEDIATELY:** Apply elution buffer onto each column to elute the labeled cells and tap **OK** immediately after.
16. Tap the Multi-24 Column Block to the MACS Elution Chamber to collect drops from the column tips and ensure complete recovery of the positive fraction.
17. **REMOVE PLATE AND COLUMNS. TOUCH OK TO END PROCESS:** Remove the Multi-24 Column Block and the lid of the MACS Elution Chamber.
18. Tap **OK** and proceed to downstream applications with the labeled target cells in the deep well plate.

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140-006-077.02

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