Anti-Nucleus MicroBeads

Order no. 130-132-997

Contents

1. Description

This product is for research use only.

Components	1.25 mL Anti-Nucleus MicroBeads: MicroBeads conjugated to monoclonal anti-nucleus antibodies
Capacity	For 25 separations each with up to 10^6 nuclei.
Product format	Anti-Nucleus MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS Separation

First, the nuclei are magnetically labeled with Anti-Nucleus MicroBeads. Then, the nucleus suspension is loaded onto a MACS Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled nuclei are retained within the column and debris runs through. After removing the column from the magnetic field, the magnetically retained nuclei are eluted as the positively selected fraction.

1.2 Background information

The Nuclei Extraction Buffer in combination with the program on the gentleMACS® Dissociator has been optimized for a gentle, rapid, and effective generation of single-nucleus suspensions from fresh or frozen tissue samples. The Anti-Nucleus MicroBeads were developed for further enrichment of the nuclei. This is necessary for some tissues, especially brain, where the nucleus suspension contains a lot of debris which might interfere with downstream applications.
The Anti-Nucleus MicroBeads have been tested on human and murine samples. The epitope they recognize is universally expressed on the nucleus surface of all cell types and species.

1.3 Applications

- Removal of debris from single-nucleus suspensions for downstream applications like single-nucleus RNA sequencing

1.4 Reagent and instrument requirements

- Nuclei Extraction Buffer (# 130-128-024)
- MACS BSA Stock Solution (# 130-091-376)
- Phosphate-buffered saline (PBS), pH 7.2
- MACS Columns and MACS Separators: Nuclei can be enriched by using LS Columns. Positive selection can also be performed by using the MultiMACS™ Cell24 Separator Plus or autoMACS Columns on the autoMACS NEO Separators.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
LS	10^8	2×10^9	MidiMACS, QuadroMACS
	10^8	10^9	MultiMACS Cell24 Separator Plus
autoMACS	2×10^8	4×10^9	autoMACS NEO Separator
Multi-24 Column Block (per column)	10^8	10^9	MultiMACS Cell24 Separator Plus

▲ **Note:** If separating with LS Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- Nucleus staining dye, e.g., 7-AAD Staining Solution (# 130-111-568), DAPI Staining Solution (# 130-111-570), or DRAQ5 Staining Solution (# 130-117-343)
- (Optional) RNase inhibitor (e.g. murine RNase Inhibitor from New England Biolabs)
- (Optional) MACS SmartStrainers (30 µm) (# 130-098-458, # 130-110-915)

2. Protocol

2.1 Preparation of nuclei separation buffer

Prepare nuclei separation buffer by diluting MACS BSA Stock Solution (10% BSA) 1:250 to reach a final BSA concentration of 0.04%, and Nuclei Extraction Buffer 1:7 (14% final concentration) in phosphate-buffered saline (PBS), pH 7.2. For example, for manual separation of 10^6 nuclei prepare a total of 10.5 mL nuclei separation buffer by adding 1.5 mL of Nuclei Extraction Buffer to 9 mL PBS and 0.04% BSA. In case of RNA-based downstream applications, add RNase inhibitor (0.2 U/µL final concentration). Keep buffer cold (+2 to +8 °C).

▲ **Note:** If only DNA-based downstream applications are to be performed, RNase inhibitor can be left out.

▲ **Note:** For automated separation using the autoMACS NEO Separator, a higher volume of nuclei separation buffer is required.

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2.2 Sample preparation

For the preparation of single-nucleus suspensions from different tissues, refer to the data sheet of the Nuclei Extraction Buffer (# 130-128-024), which is used in combination with a gentleMACS Dissociator.

2.3 Magnetic labeling

▲ Work fast, keep nuclei cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and unspecific labeling of debris.

▲ Volumes for magnetic labeling given below are for up to 10^6 nuclei. When working with fewer than 10^6 nuclei, use the same volumes as indicated. When working with higher nucleus numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 total nuclei, use twice the volume of all indicated reagent volumes and total volumes).

▲ Use a maximum of 2×10^7 nuclei per separation.

▲ If not done already during nucleus extraction, pass nuclei through 30 µm nylon mesh (e.g. MACS SmartStrainers (30 µm)) to remove cell clumps which may clog the column.

▲ The recommended incubation temperature is +2 to +8 °C. Higher temperatures and/or longer incubation times may lead to unspecific labeling. Working on ice may require increased incubation times.

1. Determine nucleus number.
2. Centrifuge nucleus suspension at 300×g for 5 minutes. Pipette off supernatant completely.
3. Resuspend nucleus pellet in 450 µL of nuclei separation buffer per 10^6 total nuclei.
4. Add 50 µL of Anti-Nucleus MicroBeads per 10^6 total nuclei.
5. Mix well and incubate for 15 minutes in the refrigerator (+2 to +8 °C).
6. Add 2 mL nuclei separation buffer (independent from nucleus sample input) and proceed to magnetic separation (2.4).

2.4 Magnetic separation

▲ Choose LS Columns and an appropriate MACS Separator.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with 3 mL of nuclei separation buffer.
3. Apply nucleus suspension onto the column. Collect flow-through containing debris.

   ▲ **Note:** Since the volume of the buffer should be kept low, add the nucleus suspension directly to the center of the column reservoir.
4. Wash column two times with 1 mL of nuclei separation buffer. Collect debris that passes through and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

   ▲ **Note:** Use buffer to rinse the inner column walls.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of nuclei separation buffer onto the column depending on nucleus sample input. Immediately flush out the magnetically labeled nuclei by firmly pushing the plunger into the column.

   For up to 4×10^6 nuclei: 1 mL

   For up to 10^7 nuclei: 2.5 mL

   For up to 2×10^7 nuclei: 5 mL

Magnetic separation with the MultiMACS Cell24 Separator Plus

▲ Refer to the the MultiMACS Cell Separator Plus user manual for instructions on how to use the MultiMACS Cell24 Separator Plus.

▲ For high-throughput nuclei separation, select program Possel_2 to collect the negative fraction as a control, followed by the positive fraction. Select program Possel_1 to isolate only the positive fraction.

2.5 Magnetic labeling and separation using autoMACS Separators

▲ Refer to the user manual and the short instructions for instructions on how to use the autoMACS Separators.

▲ Buffers used for operating the autoMACS Separators should have a temperature of ≥ +10 °C.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = unlabeled (negative) fraction,

position C = labeled (positive) fraction.

2.5.1 Magnetic labeling and separation using the autoMACS NEO Separator

▲ Replace the autoMACS Running Buffer in the system with nuclei separation buffer before use.

▲ The autoMACS NEO Separator enables stage loading to extend column capacity for selected reagents, minimizing the need to divide larger samples.

▲ For more information on selecting alternative separation programs, stage loading–compatible reagents, autolabeling-compatible reagents, and the minimal and maximal volumes for each reagent and Chill Rack, refer to www.miltenyibiotec.com/automacs-neo-sample-processing.

Magnetic separation after manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Place the Chill Rack on the MACS MiniSampler S.

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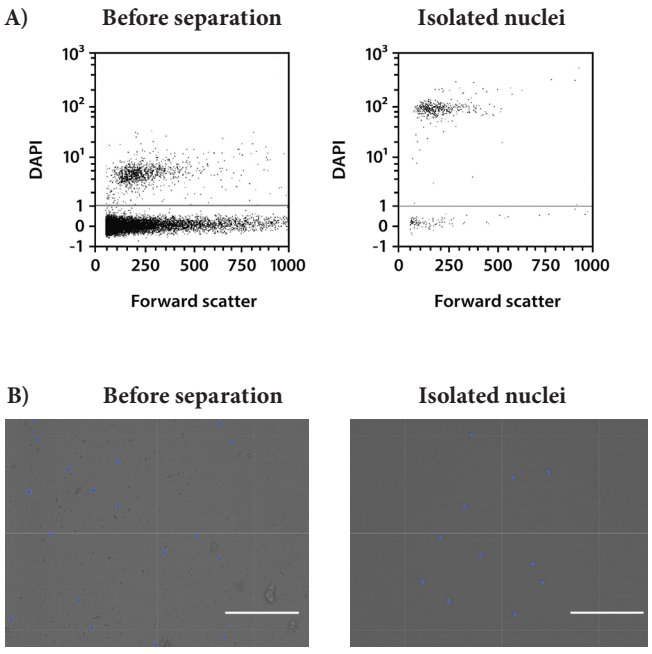
4. Select the same Chill Rack in the **Experiment** tab. An experiment is created automatically. Tap to select sample positions.
5. Assign a reagent to each sample.
6. Manual labeling is set automatically if autolabeling is not supported or no reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Manual**.
7. Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
8 The separation program for highest target cell purity is selected by default. Refer to the **Sample process** pane for all available programs.
9. Place the sample(s) and empty tubes to the Chill Rack.
10. Tap **Run** to start the separation process.

3. Example of a separation using Anti-Nucleus MicroBeads

Nuclei were extracted from mouse brain tissue using Nuclei Extraction Buffer and a gentleMACS Octo Dissociator with Heaters. They were separated using Anti-Nucleus MicroBeads, an LS Column, and a QuadroMACS™ Separator. The nuclei were stained with DAPI. Very small debris was excluded from flow cytometric analysis based on forward scatter signal. The separation of the nuclei resulted in an increase in purity from 7% to 81% (A). Bright-field microscopy images with DAPI overlay were taken before and after isolation of nuclei. The debris particles were significantly reduced after separation (B; scale = 150 µm).



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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