Anti-Nucleus MicroBeads

Order no. 130-132-997

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	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 2–8 °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
- LS Columns (# 130-042-401)
- MidiMACS™ Separator (# 130-042-302) or a QuadroMACS™ Separator
- MACS MultiStand (# 130-042-303)
- 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
- (Optional) MACS SmartStrainers (30 µm) (# 130-098-458, # 130-110-915)

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2. Protocol

2.1 Preparation of nuclei separation buffer

Prepare nuclei separation buffer by diluting MACS BSA Stock Solution 1:250 (0.04% final concentration) and Nuclei Extraction Buffer 1:7 (14% final concentration) in phosphate-buffered saline (PBS), pH 7.2. For example, for separation of 10^6 nuclei prepare 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 (2–8 °C). Degas buffer before use, as air bubbles could block the column.

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

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 2–8 °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 (2–8 °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

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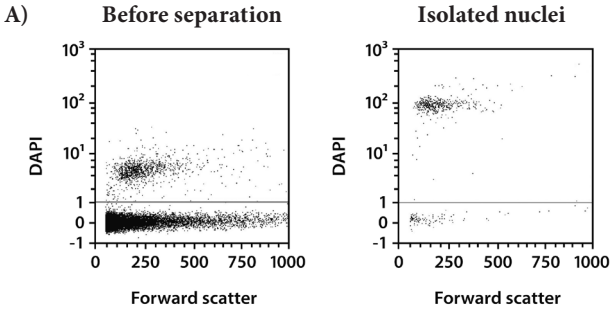
3. Example of a separation using Anti-Nucleus MicroBeads

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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