Miltenyi Biotec

Nuclei Extraction Buffer

Order no. 130-128-024

Contents

1. Description

This product is for research use only.

Components	100 mL Nuclei Extraction Buffer
Size	For 25 extractions.
Storage	Store protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the label.

1.1 Principle of the Nuclei Extraction Buffer

Tissue samples from various sources and species can be dissociated into single nuclei suspensions by combining mechanical tissue dissociation and cell lysis. The tissue sample is added to a gentleMACS™ C Tube together with the Nuclei Extraction Buffer and RNase inhibitor. The sample is dissociated using the gentleMACS Dissociator.
After dissociation, the sample is applied to a filter to remove any remaining larger particles from the single-nuclei suspension. Nuclei are collected by centrifugation and the nuclei pellet is resuspended and filtered again.

1.2 Background information

The Nuclei Extraction Buffer and the corresponding gentleMACS Program have been optimized for a gentle, rapid, and effective generation of single-nuclei suspensions from fresh and frozen tissue samples. This reliable and standardized protocol enables the recovery of high yield single-nuclei suspensions that can be used for a wide variety of studies including proteomic analysis, FACS analysis, and single-nuclei gene expression analysis.

1.3 Applications

- Dissociation of fresh, frozen (including flash-frozen and OCT embedded) tissue samples from various tissues and species into single-nuclei suspensions for subsequent molecular analyses.
- The provided protocol has been validated for a wide range of tissues, including brain, liver, heart, lung, pancreas, spleen and kidney, as well as tumor tissues including human melanoma, breast, pancreatic, colon and prostate cancer, and grafted mouse tumors.

1.4 Reagent and instrument requirements

- gentleMACS Octo Dissociator with Heaters or gentleMACS Dissociator
- gentleMACS C Tubes (# 130-093-237)
- MACS® BSA Stock Solution (# 130-091-376)
- MACS SmartStrainers (30 µm) (# 130-098-458)
- MACS SmartStrainers (70 µm) (# 130-098-462)
- (Optional) MACS SmartStrainers (100 µm) (# 130-098-463)
- (Optional) gentleMACS Octo Coolers (# 130-130-533)
- Pre-cooled centrifuge
- (Optional) RNase inhibitor (e.g. murine RNase Inhibitor from New England Biolabs)

Additional requirements for microscopic or flow cytometric analysis of single-nuclei suspensions (refer to protocol 2.3)

- Nuclei 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) Cell sorter, e.g., MACSQuant® Tyto® Sorter (# 130-103-931)

2. Protocol

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociators user manuals.

▲ Please make sure that the gentleMACS Octo Dissociator runs with the latest software update. The **4C_nuclei_1** program is included in the Miltenyi program folder from software version GM_V02.H21 (gentleMACS Octo Dissociator with Heaters) or GM_V02.R11 (gentleMACS Octo Dissociator) onwards. The latest software update can be requested at **https://www.miltenyibiotec.com/lp/2018/gentlemacs-software-update.html**.

▲ Pipette gently and slowly during all nuclei resuspension steps to minimize alteration of the extracted nuclei.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

2.1 Sample, equipment, and reagent preparation

▲ Pre-cool centrifuge, buffers, and consumables with sample contact (e.g. gentleMACS C Tube and SmartStrainers) at +4 °C.

▲ **Note:** It is recommended to pre-cool the C Tube containing prepared lysis buffer overnight at +4 °C. Ideally, perform all nuclei extraction steps in a cold room.

Preparation of lysis buffer

Per extraction add RNase inhibitor (final concentration 0.2 U/µL) to pre-cooled 4 mL Nuclei Extraction Buffer.

Preparation of nuclei separation buffer for resuspension

Prepare an appropriate amount of nuclei separation buffer for resuspension based on sample amount or tissue type (see table 1) 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, as indicated in table 2.

If further enrichment with **Anti-Nucleus MicroBeads (# 130-132-997)** is planned prepare additional nuclei separation buffer for MACS Separation. For more information refer to the respective data sheet.

Per extraction the following volumes of nuclei separation buffer for resuspension are recommended:

Amount/type of tissue	Nuclei separation buffer for resuspension
up to 50 mg	1.5 mL
50–100 mg	3 mL
100–200 mg	6 mL
Mouse brain hemisphere	8 mL

Table 1: Overview of nuclei separation buffer for resuspension volumes according to input sample.

▲ **Note:** It is strongly recommended to adjust the nuclei concentration to <5×10^6/mL to avoid nuclei aggregation.

▲ **Note:** When working with tissues with high intrinsic RNase activity (e.g. pancreas), the RNase inhibitor concentration can be increased in both prepared nuclei extraction buffer and nuclei separation buffer for resuspension in order to prevent RNA degradation.

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

▲ **Note:** Some genomic applications require the use of a different buffer for resuspension to ensure optimal performance. Follow the recommendations of the genomic application provider.

Final volume (mL)	Nuclei Extraction Buffer (mL)	PBS (ph 7.2) (mL)	MACS BSA Stock Solution (µL)
1.5	0.21	1.29	6
3.0	0.42	2.58	12
6.0	0.84	5.16	24
8.0	1.12	6.88	32

Table 2: Composition of Nuclei Extraction Buffer, PBS, and MACS BSA Stock Solution to prepare the nuclei separation buffer for resuspension.

Preparation of tissue sample

Resect tissue and cut into pieces of up to 200 mg. To store tissue, freeze them on dry ice or use liquid nitrogen.

▲ **Note:** It is recommended to cut tissue before freezing. If frozen tissue pieces are >200 mg, cut them without letting samples thaw.

▲ **Note:** In minor cases, e.g., kidney, it is recommended to cut up the tissue into pieces of <50 mg to ensure complete dissociation.

▲ **Note:** When working with OCT-embedded tissue, remove excess OCT without letting samples thaw.

2.2 Nuclei extraction protocol

▲ Optimal extraction performance depends on starting material (tissue type, amount of starting material) and downstream application and might require protocol optimization.

▲ Work fast until tissue is dissociated and keep samples on ice at all steps.

▲ Volumes given below are for up to 200 mg tissue per extraction.

1. Add 2 mL ice-cold lysis buffer to each pre-cooled gentleMACS C Tube.
2. Transfer tissue pieces to the gentleMACS C Tube containing lysis buffer and directly proceed the following steps until samples are dissociated.

   ▲ **Note:** Do not let frozen sample thaw before dissociation as endogenous RNase might degrade RNA.
3. Close gentleMACS C Tube and place it on the gentleMACS Dissociator.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.
4. Run gentleMACS Program **4C_nuclei_1** on the gentleMACS Dissociator.
5. After termination of the program, detach C Tube from the gentleMACS Dissociator and place the C Tube immediately on ice.

   ▲ **Note:** Depending on tissue type, additional 5–10 minutes incubation on ice after dissociation might be needed to optimize cell lysis. Over-lysis should be avoided as it could lead to nuclei aggregation and nuclei damage.
6. Apply nuclei suspension to a MACS SmartStrainer (70 µm) placed on a 15 mL tube.

   ▲ **Note:** For brain samples, use a MACS SmartStrainer (100 µm).

   ▲ **Note:** To increase nuclei recovery, the use of low binding tubes is recommended at this and all following steps.
7. Wash MACS SmartStrainer with 2 mL ice-cold lysis buffer.

   ▲ **Note:** Alternatively, for maximum cell recovery, rinse used C Tube with ice-cold lysis buffer before adding to the MACS SmartStrainer.
8. Discard MACS SmartStrainer and centrifuge nuclei suspension at 300×g at +4 °C for 5 minutes. Carefully aspirate supernatant completely.
9. Resuspend nuclei pellet with ice-cold nuclei separation buffer for resuspension by slowly and gently pipetting the sample up and down for 10 times.
10. Apply nuclei suspension to a MACS SmartStrainer (30 µm) placed on a 15 mL tube.

    ▲ **Note:** When working with myelin-rich brain, a myelin removal step is strongly recommended to remove myelin debris.
11. Collect nuclei suspension and proceed immediately with downstream applications, e.g., single nuclei RNA seq or nuclei sorting.

    ▲ **Note:** In case debris amount is still too high for downstream application, purify the nuclei suspension by using a cell sorter, e.g. MACSQuant Tyto.

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2.3 Labeling for microscopic or flow cytometric analysis of nuclei samples

1. Add 7-AAD Staining Solution (final concentration of 0.525 µg/mL), DAPI Staining Solution (final concentration of 0.25 µg/mL), or DRAQ5 (10 µM) Staining Solution to a small fraction of the nuclei suspension. Mix by inverting gently.
2. Incubate 5 minutes at +4 °C.
3. Load sample on a hemocytometer or on a flow cytometer, e.g., MACSQuant Analyzer.
4. Analyze sample according to manufacturer's recommendation.

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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