Miltenyi Biotec

FFPE Tissue Dissociation Kit for RNA Profiling

Order no. 130-134-089

Contents

1. Description

This product is for research use only.

Components	**1 vial of Enzyme D (lyophilized powder)** **2.5 mL Enzyme P** **13 mL Buffer S (20×)**
Size	For 25 digestions. The specified number of digestions is valid when digesting up to two 25 µm tissue scrolls in 2.5 mL enzyme mix following the protocol in chapter 2.2.
Storage	Upon arrival store Enzyme P aliquoted at –20 °C. Store all other components at +2 to +8 °C. Reconstitute all components before the date indicated on the box label. For information about reconstitution and storage after reconstitution of the lyophilized components refer to chapter 2.1.

1.1 Principle of the FFPE Tissue Dissociation Kit for RNA Profiling

Formalin-fixed paraffin-embedded (FFPE) tissue sections from various tissues and species, for example, human tumor, skin, or lymph node samples, are dissociated into single-nucleus suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular matrix. This maintains the structural integrity of tissues. The FFPE tissue sections are deparaffinized and rehydrated. Then, samples are dissociated using the kit components and the gentleMACS™ Octo Dissociator with Heaters. After dissociation, the sample is applied to a filter to remove any remaining larger particles from the single-nucleus suspension.

1.2 Background information

The FFPE Tissue Dissociation Kit for RNA Profiling has been optimized for the gentle, rapid, and effective generation of single-nucleus suspensions from FFPE tissue samples. Thus, a high yield of single-nuclei can be obtained. Extracted nuclei can be subsequently counted by flow cytometry or microscopy and be used for molecular applications like single-nucleus RNA sequencing.

1.3 Applications

- Dissociation of FFPE tissue sections from various tissues into single-nucleus suspensions for subsequent single-nucleus RNA sequencing
- Enumeration of nuclei by flow cytometry or fluorescence microscopy

1.4 Reagent and instrument requirements

- gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (25 tubes (# 130-093-237))
- Pre-Separation Filters (30 µm) (# 130-041-407)
- Centrifuge (cooled to +4 °C)
- Glass Pasteur pipette
- Xylol or xylol substitute
- 50%, 70%, and 100% ethanol
- Sterile, RNase-free distilled water
- PBS
- 1 M RNase-free Tris, pH 8
- 10% RNase-free BSA Stock Solution
- RNase inhibitor (40 U/µL)

2. Protocol

▲ For details on the use of the gentleMACS Octo Dissociator with Heaters, refer to the gentleMACS Dissociators user manuals.

▲ Volumes given below are for up to two FFPE tissue scrolls (25 µm each) in 2.5 mL enzyme mix per gentleMACS C Tube.

▲ Separation of tumor cells from healthy cells, e.g., with Anti-Cytokeratin MicroBeads, human (# 130-123-094), requires a specific antigen retrieval step (incubation at +80 °C) and is not part of this protocol. For this application, use the FFPE Tissue Dissociation Kit (# 130-188-052) and refer to the respective protocol.

2.1 Reagent preparation

▲ Handle all buffers and reconstituted enzymes under sterile conditions to avoid contamination.

▲ Do not prepare a stock solution containing both Enzyme D and Enzyme P.

1. Prepare Buffer S (1×) by mixing, for example, 500 µL Buffer S (20×) with 9.5 mL sterile distilled water under sterile conditions. For long-term storage, store Buffer S (1×) at +2 to +8 °C.
2. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL of Buffer S (1×). Invert vial after closing and wait 5–10 minutes while inverting every minute to

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

140-006-964.01

dissolve the pellet. Do not resuspend by pipetting or vortexing. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

3. Prepare aliquots of Enzyme P of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months.

4. Prepare resuspension buffer for dissociated FFPE samples (550 µL per dissociation run), e.g., 0.5× PBS containing 50 mM RNase-free Tris, pH 8, 0.02% RNase-free BSA, and 0.2 U/µL RNase inhibitor. Table 1 shows the required volumes of the respective stock solutions for one or four preparations. An overfill of 10% is included. Adapt the calculations for the target concentrations if working with other stock solutions.

   ▲ **Note:** This kit has been validated using the indicated resuspension buffer. Use of any other resuspension buffer is at own risk.

	For one dissociation run	For four dissociation runs
1× PBS	275 µL	1100 µL
1 M RNase-free Tris, pH 8	28 µL	112 µL
10% RNase-free BSA	1 µL	4 µL
RNase inhibitor (40 U/µL)	3 µL	12 µL
Sterile, RNase-free water	243 µL	972 µL

Table 1: Preparation of resuspension buffer for one dissociation run or four dissociation runs.

2.2 Dissociation of FFPE tissue

▲ The volumes listed below are based on one dissocation run of up to two FFPE tissue scrolls. Increase the volumes based on the number of dissociation runs. Use a maximum of two FFPE tissue scolls per C Tube.

▲ Cool down centrifuge to +4 °C.

▲ Prewarm an aliquot of 2.35 mL Buffer S (1×) for at least 10 min at +37 °C before use for step 9.

▲ Place an aliquot of 1 mL Buffer S (1×) on ice.

▲ This protocol has been validated with xylene. Use xylene substitutes at own risk.

▲ Always use glass Pasteur pipettes as samples may stick to plastic pipettes.

1. Prepare up to two 25 µm tissue scrolls from an FFPE block, remove excessive paraffin, and transfer the scrolls into a gentleMACS C Tube.

   ▲ **Note:** The yield from two 25 µm scrolls may not be sufficient due to differences in cell density and tissue size in the FFPE block. If the final number of nuclei is not sufficient, pooling of multiple runs is needed. It is recommended to perform a test run to determine the number of scrolls required for a specific target nuclei number.

   ▲ **Note:** In case a tissue section is already mounted on a slide, place the slide including the section in an appropriate container for deparaffination and rehydration (steps 2–8). Be aware that much higher volumes are needed to wet samples.

2. Add 3 mL of xylene or xylene substitute to the gentleMACS C Tube, incubate for 10 minutes at room temperature, and aspirate liquid completely using a glass Pasteur pipette.

   ▲ **Note:** For optimal aspiration of liquids, move the section(s) carefully with the glass Pasteur pipette tip along the tube wall above the liquid level, then aspirate the liquid completely without touching or removing the scrolls.

3. Repeat step 2 twice for a total of three washes with xylene or xylene substitute.

4. Add 3 mL of 100% ethanol to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid completely using a glass Pasteur pipette.

   ▲ **Note:** For optimal aspiration of liquids, move the section(s) carefully with the glass Pasteur pipette tip along the tube wall above the liquid level, then aspirate the liquid completely without touching or removing the scrolls.

5. Add 1 mL of 100% ethanol to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid completely using a glass Pasteur pipette.

   ▲ **Note:** For optimal aspiration of liquids, move the section(s) carefully with the glass Pasteur pipette tip along the tube wall above the liquid level, then aspirate the liquid completely without touching or removing the scrolls.

6. Add 1 mL of 70% ethanol to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid completely using a glass Pasteur pipette.

   ▲ **Note:** For optimal aspiration of liquids, move the section(s) carefully with the glass Pasteur pipette tip along the tube wall above the liquid level, then aspirate the liquid completely without touching or removing the scrolls.

7. Add 1 mL of 50% ethanol to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid completely using a glass Pasteur pipette.

   ▲ **Note:** For optimal aspiration of liquids, move the section(s) carefully with the glass Pasteur pipette tip along the tube wall above the liquid level, then aspirate the liquid completely without touching or removing the scrolls.

8 Add 1 mL of deionized water to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid completely using a glass Pasteur pipette.

9. Prepare enzyme mix by mixing 2.35 mL of prewarmed 1× Buffer S, 100 µL of Enzyme D, and 65 µL of Enzyme P. Transfer dissociation mix into the gentleMACS C Tube and close it tightly.

10. Attach the C Tube to the gentleMACS Octo Dissociator with Heaters and place the Heater Unit on the C Tube. Run the gentleMACS program 37C_FFPE_1.

11. After termination of the program, detach the C Tube from the gentleMACS Octo Dissociator with Heaters and place it on ice.

12. (Optional) Perform a short spin up to 300×g to collect the sample at the bottom of the tube. Resuspend the cells.

13. Apply the cell suspension to a Pre-Separation Filter (30 µm) placed on a 15 mL tube on ice.

14. Rinse the used C Tube with 1 mL ice-cold 1× Buffer S and apply to the Pre-Separation Filter (30 µm).

15. Discard the filter and centrifuge the nuclei suspension at 850×g at +4 °C for 5 minutes. Aspirate supernatant completely.

16. Resuspend the nuclei pellet in 0.5 mL of an appropriate resuspension buffer for downstream application.

    ▲ **Note:** A nuclei pellet might not be visible due to the low amount of nuclei.

17. Process nuclei immediately for further applications, e.g., nuclei counting or single-nucleus RNA sequencing.

140-006-964.01

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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

gentleMACS and the Miltenyi Biotec logo are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this document are the property of their respective owners and are used for identification purposes only.