Miltenyi Biotec

FFPE Tissue Dissociation Kit

Order no. 130-118-052

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	6 vials, containing: **1 vial of Enzyme D (lyophilized powder)** **1 vial of Enzyme G (lyophilized powder)** **100 mL of Buffer U** **100 mL of Buffer V** **100 mL of Buffer W** **100 µL of Reagent A**
Size	For 25 digestions. The specified number of digestions is valid when digesting up to two 50 µm sections in 2.5 mL enzyme mix following the protocol in chapter 2.2.
Product format	Buffer V and Buffer W contain 0.09% sodium azide.
Storage	Upon arrival store all components at 2–8 °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

Formalin-fixed paraffin-embedded (FFPE) tissue sections from various tissues and species, for example, human tumor, skin, or lymph node samples, can be dissociated into single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular matrix, which maintains the structural integrity of tissues.

The FFPE tissue sections are deparaffinized. After a defixation step, samples are dissociated using the kit components and the gentleMACS™ Dissociator with Heaters. After dissociation, the sample is applied to a filter to remove any remaining larger particles from the single-cell suspension.

1.2 Background information

The FFPE Tissue Dissociation Kit has been optimized for the gentle, rapid, and effective generation of single-cell suspensions from human FFPE carcinoma sections. Thus, a high yield of single-cells can be obtained, while preserving the important cell epitopes cytokeratin for cell analysis of carcinoma cells and vimentin for non-carcinoma cells.

Dissociated cells can be subsequently separated by flow sorting or analyzed by flow cytometry *in vitro* for phenotype distributions, and other genetic or proteomic studies.

1.3 Applications

- Dissociation of FFPE tissue sections from various tissues into single-cell suspensions for subsequent cell separation using flow sorting technologies.
- For application of the kit with the 10x Genomics Chromium Single Cell Fixed RNA Profiling Assay, download the demonstrated protocol for use with FFPE tissue at the manufacturer's website[1].
- Phenotyping or enumeration of cells by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- Sterile distilled water.
- Water bath or incubator (pre-heated to 80 °C).
- Centrifuge (cooled to 4 °C).
- 50%, 70%, and 100% ethanol.
- Xylol.
- Glass pasteur pipette.
- Double-distilled water.
- Pre-Separation Filters (70 µm) (# 130-095-823).
- gentleMACS Octo Dissociator with Heaters (# 130-096-427).
- gentleMACS C Tubes (25 tubes (# 130-093-237), 4×25 tubes (# 130-096-334)).

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- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Cytokeratin Antibody, anti-human, FITC, REAfinity™ (clone: REA831), Vimentin Antibody, anti-human, APC, REAfinity (clone: REA409), and CD235a (Glycophorin A) Antibody, anti-human, PE, REAfinity (clone: REA175). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Pre-Separation Filters (20 µm) (# 130-101-812).
- (Optional) Nuclei staining dye, e.g., DAPI Staining Solution (# 130-111-570).

  ▲ **Note:** Prepare DAPI Staining Solution dilution with Buffer W.

2. Protocol

▲ For application of the kit with the 10x Genomics Chromium Single Cell Fixed RNA Profiling Assay, do not follow the instructions in this data sheet, but download the demonstrated protocol for use with FFPE tissue at the manufacturer's website[1].

▲ 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 sections (50 µm each) in 2.5 mL dissociation mix per C Tube.

2.1 Reagent preparation

1. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL sterile distilled water. Do not try to resuspend by pipetting or vortexing. Invert vial after closing and wait 5–10 minutes while inverting every minute to dissolve the pellet. 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.
2. Prepare Enzyme G by reconstitution of the lyophilized powder in the vial with 3 mL sterile distilled water. Do not try to resuspend by pipetting or vortexing. Invert vial after closing and wait 5–10 minutes while inverting every minute to dissolve the pellet. 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.

2.2 Dissociation of FFPE tissue

▲ Pre-heat incubator and 3 mL of Buffer U to 80 °C for step 10. Cool down centrifuge and Buffer W to 4 °C.

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

1. Prepare up to two 50 µm sections from a FFPE block and transfer them into a gentleMACS C Tube.

   ▲ **Note:** Discard the first section from the FFPE block.
2. Add 3 mL of xylol to the gentleMACS C Tube, incubate for 10 minutes at room temperature, and aspirate liquid using a pasteur pipette.
3. Repeat step 2 two times.
4. Add 3 mL of 100% ethanol to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid using a pasteur pipette.
5. Add 1 mL of 100% ethanol to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid using a pasteur pipette.
6. Add 1 mL of 70% ethanol to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid using a pasteur pipette.
7. Add 1 mL of 50% ethanol to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid using a pasteur pipette.
8. Add 1 mL of double-distilled water to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid using a pasteur pipette.
9. Add 1 mL of non–pre-heated Buffer U to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid using a pasteur pipette.
10. Add 3 mL of the pre-heated Buffer U to the gentleMACS C Tube. Place the tube in the pre-heated water bath or incubator and incubate for 75 minutes at 80 °C.
11. Take samples out of the incubator, let them cool down for 20 minutes, and aspirate liquid using a pasteur pipette.
12. Add 1 mL of the Buffer V to the gentleMACS C Tube, incubate for 30 seconds at room temperature, and aspirate liquid using a pasteur pipette.
13. Prepare dissociation mix by mixing 2.3 mL Buffer V, 100 µL of dissolved Enzyme D, 100 µL of dissolved Enzyme G, and 2.5 µL of Reagent A. Transfer it to the gentleMACS C Tube and tightly close it.

    ▲ **Note:** Close C Tube tightly beyond the first resistance.

    ▲ **Note:** When working with multiple samples, prepare a master mix with appropriate volumes of Buffer V, dissolved Enzyme D, and dissolved Enzyme G. Aliquot the master mix and add Reagent A to every sample separately.
14. Run the gentleMACS Program **37C_FFPE_1** on the gentleMACS Octo Dissociator with Heaters.
15. After termination of the program, detach C Tube from the gentleMACS Octo Dissociator with Heaters and place the C Tube on ice.
16. (Optional) Perform a short spin up to 300×g to collect the sample at the bottom of the tube. Resuspend the cells.
17. Apply the cell suspension to a Pre-Separation Filter (70 µm) placed on a 15 mL tube on ice.
18. Wash the filter with 1 mL ice-cold Buffer W.

    ▲ **Note:** Alternatively, for maximum cell recovery, rinse the used C Tube with the 1 mL ice-cold Buffer W before adding it to the filter.
19. Discard the filter and centrifuge cell suspension at 1000×g at 4 °C for 5 minutes. Aspirate supernatant completely.
20. Wash tube with 1 mL ice-cold Buffer W.
21. Centrifuge cell suspension at 1000×g at 4 °C for 5 minutes. Aspirate supernatant completely.
22. Resuspend cell pellet with max. 0.5 mL ice-cold Buffer W to the required volume for further applications.

    ▲ **Note:** A cell pellet might not be visible due to the low amount of cells.
23. Process cells for further applications, e.g., flow cytometric analysis of human carcinoma samples (refer to 2.3).

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2.3 Immunofluorescent staining

1. Add fluorochrome-conjugated antibodies, e.g., Cytokeratin Antibody-FITC, Vimentin Antibody-APC, and CD235a (Glycophorin A)-PE, twice as concentrated as recommended by manufacturer to the resuspended sample.
2. Incubate 10 minutes at 4 °C.
3. Add 1 mL Buffer W and centrifuge at 1000×g at 4 °C for 5 minutes. Aspirate supernatant completely.
4. Resuspend cell pellet in a nuclei staining dye, e.g., add 500 µL DAPI Staining Solution (2 µg/mL) and incubate for 30 minutes at 4 °C.

   ▲ **Note:** Prepare DAPI Staining Solution dilution with Buffer W.
5. Centrifuge at 1000×g at 4 °C for 5 minutes. Aspirate supernatant completely.
6. Resuspend sample in max. 250 µL of ice-cold Buffer W and apply the cell suspension to a Pre-Separation Filter (20 µm) placed on an appropriate tube.
7. Wash the Pre-Separation Filter (20 µm) with 250 µL Buffer W and immediately proceed with flow cytometric analysis.

3. References

1. https://pages.10xgenomics.com/TCH-2022-09-DOWNLOAD-P_SFRP-SCFFPE-DP-SOFT-LAUNCH_GatedLP.html

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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