Miltenyi Biotec

Spleen Dissociation Kit
mouse

Order no. 130-095-926

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1. Description

Components	3 vials, containing: **13 mL of Buffer S (20× Stock Solution)** **1 vial of Enzyme D (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)**
Size	For 50 digestions. The specified number of digestions is valid when digesting a spleen with an average weight of 80–140 mg following the protocol in chapter 2.2.
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 Spleen Dissociation Kit

Spleen 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 spleen is enzymatically digested using the kit components, and the gentleMACS™ Dissociators are used for the mechanical dissociation steps. After dissociation, the sample is applied to a filter to remove any remaining larger particles from the single-cell suspension.
Cells should be processed immediately for downstream applications, such as cell separation, cell culture, cellular or molecular analyses.

1.2 Background information

The Spleen Dissociation Kit, mouse has been designed for the gentle, rapid, and efficient generation of single-cell suspensions from mouse spleen. It is optimized for a high yield of leukocytes, especially dendritic cells, while preserving all cell surface epitopes. Dissociated cells can be isolated using MACS® Technology. Furthermore, the single-cell suspension can be analyzed *in vitro* for phenotype distributions, and other functional, genetic, or proteomic studies performed.

1.3 Applications

- Dissociation of mouse spleen into single-cell suspensions for subsequent isolation of dendritic cells using CD11c MicroBeads, mouse (# 130-052-001), Anti-mPDCA-1 MicroBeads, mouse (# 130-091-965), or Pan DC MicroBeads, mouse (# 130-092-465).
- Cultivation of spleen cell populations.
- Phenotyping or enumeration of spleen cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- PBS: phosphate-buffered saline pH 7.2
- PEB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Always use freshly prepared buffer. Do not use autoMACS® Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.
- Pre-Separation Filters, 30 µm (# 130-041-407)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C
- gentleMACS Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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2. Protocol

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

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ One mouse spleen is dissociated in a volume of approximately 2.5 mL enzyme mix. The weight of one mouse spleen amounts to 80–140 mg (female BALB/c mouse, 6–7 weeks old).

▲ Remove fat tissue from the dissected mouse spleen before dissociation.

2.1 Reagent preparation

1. Prepare 1× Buffer S by adding, for example, 1 mL of 20× Buffer S aseptically to 19 mL of sterile, distilled water. Store at 2–8 °C.
   ▲ **Note:** Handle under sterile conditions.
2. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL of 1× Buffer S. 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. For cell culture experiments subsequent to tissue dissociation, Enzyme D should be sterile filtered prior to aliquoting.
3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL 1× Buffer S. Do not vortex. 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 Spleen dissociation protocol

1. Prepare enzyme mix by adding 2.4 mL of 1× Buffer S, 50 µL of Enzyme D, and 15 µL of Enzyme A into a gentleMACS C Tube.
2. Transfer one mouse spleen into the gentleMACS C Tube containing the enzyme mix.
3. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
   ▲ **Note:** Close C Tube tightly beyond the first resistance.
   ▲ **Note:** It has to be ensured that the sample material is located in the area of the rotor/stator.
4. Run the gentleMACS Program **m_spleen_02**.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_m_SDK_1** and continue with step 9.
5. After termination of the program, detach C Tube from the gentleMACS Dissociator.
   ▲ **Note:** The spleen will not be completely dissociated after this step. In the unexpected event that the spleen is not dissociated at all, repeat steps 4 and 5.
6. Incubate sample for 15 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
   ▲ **Note:** Operate MACSmix Tube Rotator on permanent run at a speed of approximately 12 rpm.
7. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.
   ▲ **Note:** It has to be ensured that the sample material is located in the area of the rotor/stator.
8. Run the gentleMACS Program **m_spleen_03**.
9. After termination of the program, detach C Tube from the gentleMACS Dissociator.
10. (Optional) Perform a short centrifugation step to collect the sample material at the tube bottom.
11. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 30 µm, placed on a 15 mL tube.
    ▲ **Note:** Dissociated tissue can be removed from the closed C Tube by pipetting through the septum-sealed opening in the center of the cap of the C Tube. Use ART 1000 REACH 1000 µL pipette tips.
12. Wash the filter with 2.5 mL 1× Buffer S.
13. Discard the filter and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
14. Resuspend cells with medium or an appropriate buffer to the required volume for further applications. For example, resuspend cells in PEB buffer for magnetic cell separatiom or flow cytometry.
15. Process cells immediately for further applications.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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