Miltenyi Biotec

Neonatal Heart Dissociation Kit
mouse and rat

Order no. 130-098-373

Contents

1. Description

This product is for research use only.

Components	7 vials, containing: **2.5 mL of Enzyme P** **2×50 mL of Buffer X** **1.5 mL of Buffer Y** **1 vial of Enzyme A (lyophilized powder)** **1 mL of Buffer A** **1 vial of Enzyme D (lyophilized powder)**
Size	For 25 digestions of 2.5 mL.
Storage	Upon arrival immediately store Enzyme P in aliquots at –20 °C. Store all other components at 2–8 °C upon arrival. Reconstitute Enzymes A and D 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 MACS® Separation

Neonatal hearts from mice and rats 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 neonatal hearts are enzymatically digested using the kit components and the gentleMACS™ Dissociator is 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

Neonatal cardiomyocytes are widely used to study and understand the morphological, biochemical, and electrophysiological characteristics of the heart based on a broad spectrum of experiments, such as studies of contraction, ischaemia, hypoxia, and the toxicity of various compounds.
The Neonatal Heart Dissociation Kit, mouse and rat has been designed for the gentle, rapid, and effective generation of single-cell suspensions from mouse and rat hearts. It is optimized for a high yield of viable cardiomyocytes as well as non-myocytes, e.g., cardiac fibroblasts and endothelial cells. The single-cell suspension can be cultured and utilized for functional, genetic, or molecular studies.

1.3 Applications

- Dissociation of mouse and rat neonatal hearts into single-cell suspensions.
- Single-cell suspensions derived from neonatal hearts can be used for:
  - Purification of cardiomyocytes.
  - Enumeration and phenotyping of individual cardiac cell populations by flow cytometry or fluorescence microscopy.
  - Cultivation of beating cardiomyocytes or non-myocytes like cardiac fibroblasts and endothelial cells.

1.4 Reagent and instrument requirements

- Phosphate-buffered saline (PBS), pH 7.4
- Cell culture medium without fetal bovine serum (FBS), e.g., DMEM
- Cell culture medium with FBS
- MACS SmartStrainers (70 µm) (# 130-098-462)
- 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)
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- PEB buffer: Prepare a solution containing PBS, pH 7.4, 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.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse or rat serum albumin, mouse or rat serum, or FBS. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

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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.

2.1 Reagent and instrument preparation

1. Enzyme P is ready to use. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months.
2. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL Buffer A supplied with the kit. 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.
3. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL of cell culture medium without FBS, e.g., DMEM. 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.

Enzyme mix 1		Enzyme mix 2		
Enzyme P	Buffer X	Buffer Y	Enzyme A	Enzyme D
62.5 µL	2300 µL	25 µL	12.5 µL	100 µL

2.2 Neonatal heart dissociation protocol

▲ For mouse and rat postnatal day 0–3 (P0–P3).

▲ Volumes given below are for up to 20 neonatal mouse hearts or up to 10 neonatal rat hearts per C Tube and 2.5 mL of enzyme mix. When working with more hearts, scale up all reagent volumes and total volumes accordingly. Up to 20 neonatal rat hearts can be dissociated per C Tube with 5 mL of enzyme mix.

1. For mouse:
   Harvest neonatal mouse hearts and transfer into a 10 cm dish containing PBS. Utilizing forceps, carefully pump remaining blood out of the hearts. Cut vessels and remaining connective tissue away from the ventricles.

   For rat:
   Harvest neonatal rat hearts and transfer into a 10 cm dish containing PBS. Cut vessels and remaining connective tissue away from the ventricles. Cut each heart into small pieces (1–2 mm^3).
2. Preheat enzyme mix 1 for 5 minutes at 37 °C.

   ▲ **Note:** Preheating is not required if using the heating function of the gentleMACS Octo Dissociator with Heaters.
3. Add 2362.5 µL of enzyme mix 1 to 137.5 µL of enzyme mix 2.
4. Transfer harvested tissue into the gentleMACS C Tube.

   ▲ **Note:** To reduce the volume of washing medium within the tube let tissue settle down by gravity and remove supernatant carefully.
5. Add 2.5 mL of enzyme mix, tightly close the C Tube.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.
6. Invert C Tube and place it with the cap down. To maximize cell recovery the C Tube should remain in this orientation until step 11.
7. (Optional) If using the heating function of the gentleMACS Octo Dissociator with Heaters attach C Tube upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters. Run program **37C_mr_NHDK_1** and continue with step 12.
8. Incubate sample without agitation for 15 minutes at 37 °C.
9. Attach C Tube onto the sleeve of the gentleMACS Dissociator.
10. Run the gentleMACS Program **mr_neoheart_01**.
11. Repeat steps 8–10 two times.
12. After termination of the program, detach C Tube from the gentleMACS Dissociator and add 7.5 mL of cell culture medium with FBS.
13. Resuspend sample and apply the cell suspension to a MACS SmartStrainer (70 µm) placed on a suitable tube.
14. Wash MACS SmartStrainer (70 µm) with 3 mL of cell culture medium with FBS.
15. Discard filter and centrifuge cell suspension at 600×g for 5 minutes. Aspirate supernatant completely.
16. Proceed with a red blood cell lysis (refer to section 2.3).

2.3 Red blood cell lysis

1. Resuspend cell pellet in 1 mL of PEB buffer and add 10 mL of 1× Red Blood Cell Lysis Solution to remove erythrocytes.
2. Incubate for maximal 2 minutes at room temperature (19–25 °C).
3. Centrifuge at 600×g for 5 minutes. Aspirate supernatant completely.
4. Add 15 µL of Enzyme A to 10 mL PBS in a fresh tube.
5. Resuspend cell pellet in 10 mL PBS containing Enzyme A.
6. Centrifuge at 600×g for 5 minutes. Aspirate supernatant completely.
7. Resuspend cells with appropriate buffer or medium to the required volume for further applications.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols.