Miltenyi Biotec

Heart Perfusion Kit
mouse

Order no. 130-134-266

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1. Description

This product is for research use only.

Components	**2 vials of Enzyme D**, lyophilized powder **1 vial of Enzyme T**, lyophilized powder **1 vial of Enzyme A**, lyophilized powder **100 mL Buffer H (20×)** **1 mL Reagent C** **1 mL Reagent E** **Sterile surgical thread (10×50 cm)**
Capacity	For 25 digestions. The specified number of digestions is valid for digesting of one mouse heart per perfusion following the protocol in chapter 2.2.
Storage	Upon arrival store the enzymes at +2 to +8 °C. Store other components at room temperature. Reconstitute the enzymes before the date indicated on the box label. For information about reconstitution and storage after reconstitution refer to chapter 2.1.

1.1 Principle of the Heart Perfusion Kit, mouse

By using the gentleMACS™ Perfusion Technology, mouse heart tissue can be dissociated into highly viable single-cell suspensions. The perfusion is performed on the gentleMACS Octo Dissociator with Heaters equipped with gentleMACS Perfusion Sleeves in combination with gentleMACS Perfusers 2. Mouse heart is enzymatically digested using the components of the Heart Perfusion Kit, mouse. It loosens the structural integrity of the extracellular matrix in the tissue during the perfusion process. Afterwards, single cells are liberated from the tissue by a short mechanical disruption of the perfused tissue using the gentleMACS Octo Dissociator with Heaters and a gentleMACS C Tube. The sample is then applied to a MACS® SmartStrainer 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, and cellular or molecular analyses.

1.2 Background information

The Heart Perfusion Kit, mouse has been developed for the gentle, rapid, and efficient generation of single-cell suspensions from mouse heart in combination with the gentleMACS Perfusion Technology. It is optimized for a high yield of parenchymal mouse heart cells, i.e., cardiomyocytes. Dissociated cells can subsequently be cultured or non-parenchymal cells can be isolated using MACS Technology. Furthermore, single-cell suspensions can be phenotyped and other functional, genetic, or proteomic studies can be performed.

1.3 Applications

- Dissociation of mouse heart for subsequent functional assays
- Cultivation of cardiomyocytes
- Phenotyping or enumeration of cells by flow cytometry or fluorescence microscopy
- Cell isolation using MACS Technology

1.4 Reagent and instrument requirements

- gentleMACS Octo Dissociator with Heaters
- gentleMACS Perfusion Sleeves (# 130-128-752)
- gentleMACS Perfusers 2 (# 130-134-803)
- gentleMACS C Tubes (25 tubes, # 130-093-237; 4×25 tubes, # 130-096-334)
- MACS SmartStrainers (100 µm) (50 filters, # 130-098-463; 4×25 filters, # 130-110-917)
- MACS BSA Stock Solution (# 130-091-376)
- Muscle myosin-II inhibitor, e.g., 2,3 Butandione monoxime (BDM)
- Disposable glass Pasteur pipettes with elongated tips (230 mm)
- Water bath
- Liquid suction pump
- Cooling centrifuge with a swinging bucket rotor
- Petri dishes (diameter: 6 cm)
- 15 and 50 mL reagent tubes
- Surgical instrument set
- (Optional) MACS Tissue Storage Solution (# 130-100-008)

140-006-868.02

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2. Protocol

The perfusion process takes place in the gentleMACS Perfuser 2. The gentleMACS Perfuser 2 (figure 1) is pre-assembled and consists of the two main parts: the lid-clamp-grid assembly and the base.

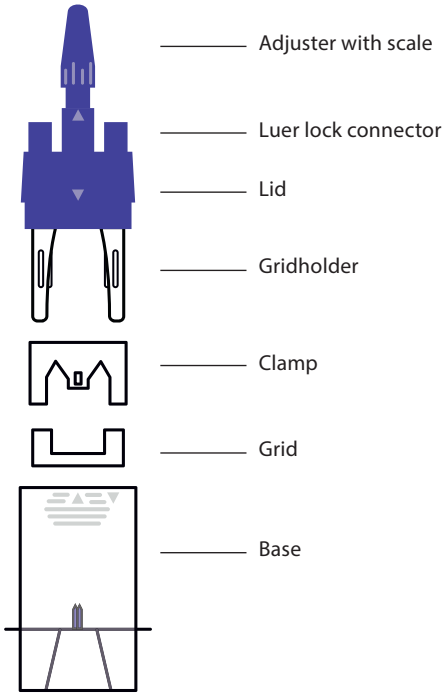


Figure 1: Overview of the gentleMACS Perfuser 2.

▲ For euthanizing mice, apply the recommendation of the American Veterinary Medical Association (AVMA) guidelines using a 30–70% CO_2 flow rate per minute.

▲ A minimum heart size is essential for good perfusion results. It is recommended to work with mice with an age of at least 4 weeks for the CD1 mouse strain or 5 weeks for the C57BL/6 or Balb/c mouse strains.

▲ For details on the use of gentleMACS Dissociators, refer to the respective user manual and www.miltenyibiotec.com/gentlemacs.

▲ For details on the use of the gentleMACS Perfusers 2 and the gentleMACS Perfusion Sleeves, refer to the respective data sheet.

▲ Make sure that the gentleMACS Octo Dissociator with Heaters runs with the latest software update and the required programs 37C_m_HPK_1 and HPK_CR_1 are installed.

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

▲ Refer to the Miltenyi YouTube channel for the detailed tutorial video "Isolating viable primary cardiomyocytes with gentleMACS™ Perfusion Technology."

2.1 Reagent preparation

▲ Buffer H (20×) is susceptible to bacterial contamination. Sterile aliquoting is recommended.

▲ Pre-warm Buffer H (20×) at +37 °C for at least 45 minutes before first use.

▲ Shake Buffer H (20×) thoroughly before use.

Preparation of reconstitution buffer

Prepare reconstitution buffer by diluting 500 µL Buffer H (20×) with 9.5 mL sterile water. Add 1 µL Reagent C and mix well.

Enzyme reconstitution

1. Prepare Enzyme D by reconstitution of the lyophilized powder in the two vials with 3 mL of reconstitution buffer per vial. Close the lid and invert the vial. Wait for 5–10 minutes for the powder to dissolve. Do not pipette up and down. Prepare aliquots 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, sterile-filter Enzyme D prior to aliquoting.

   ▲ **Note:** Make sure to thoroughly mix the enzyme by inverting the vial immediately before taking out the required reaction volume.

2. Prepare Enzyme T by reconstitution of the lyophilized powder in the vial with 3 mL of reconstitution buffer. Close the lid and invert the vial. Wait for 5–10 minutes for the powder to dissolve. Do not pipette up and down. Prepare aliquots to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

   ▲ **Note:** Make sure to thoroughly mix the enzyme by inverting the vial immediately before taking out the required reaction volume.

3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 0.3 mL of sterile water. Do not vortex. Prepare aliquots to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

   ▲ **Note:** Make sure to thoroughly mix the enzyme by inverting the vial immediately before taking out the required reaction volume.

Preparation of pre-digestion buffer, equlibration buffer, and stop buffer

▲ Volumes indicated below are for one perfusion. When performing more perfusions, upscale volumes accordingly.

▲ Muscle myosin-II inhibitors are used to extend the lifespan of cardiomyocytes by inhibiting contraction. For experiments with cardiomyocytes where contraction is required, do not use muscle myosin-II inhibitors and skip preparation steps 2 and 3

1. Prepare 60 mL Buffer H (1×) by diluting 3 mL Buffer H (20×) with 57 mL sterile, distilled water. Buffer H (1×) is used as the basis to prepare the perfusion buffers.
2. Prepare 4 mL muscle myosin-II inhibitor solution, e.g., a 0.5 M BDM solution by dissolving 0.2 g BDM in 4 mL Buffer H (1×). Incubate for 10–20 minutes in a +37 °C water bath until the BDM is dissolved. Shake every 5 minutes during the incubation.
3. Prepare pre-digestion buffer by mixing 56 mL Buffer H (1×) with 3 mL muscle myosin-II inhibitor solution, e.g., 0.5 M BDM solution.
4. Pre-warm pre-digestion buffer at +37 °C.
5. Prepare equilibration buffer and stop buffer according to table 1 and adjust to the respective temperature.

	Equilibration buffer	Stop buffer
Pre-digestion buffer	15 mL	10 mL
Reagent C	1.5 µL	–
BSA	–	0.5 mL
Temperature	+37 °C	on ice

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Table 1: Preparation of equlibration buffer and stop buffer.

6. Take 6 mL of the prepared equilibration buffer and transfer to a separate tube. This is used to prepare the enzyme digestion mix. Enzymes are added freshly shortly before the enzyme digestion mix is used (chapter 2.2, step 20).

2.2 Heart perfusion

▲ It is strongly recommended that the entire workflow is carried out without interruption. If this is not possible, e.g., due to short-term transport to another laboratory, perform at least steps 1–10 until the heart is ligated and resected. Use cold MACS Tissue Storage Solution supplemented with Reagent E (1:200) for transport of resected and ligated hearts only. A longer processing time will impact cell yield and viability.

▲ Only the whole mouse heart can be perfused. Do not damage the heart prior to perfusion.

▲ gentleMACS Perfusers 2 are readily adjusted for perfusion of mouse hearts. If the position of the adjuster has been altered by accident, it can be re-adjusted to the original position by turning it clockwise to the lowest position and then slightly back until the "5" on the adjuster's scale matches the arrow head on the adjuster. Now turn the adjuster again by rotating it counterclockwise by 720°.

▲ Ensure that the regular gentleMACS Sleeves have been exchanged by the Perfusion Sleeves before attaching the gentleMACS Perfuser 2.

1. Pre-heat a water bath at +37 °C and cool a centrifuge to +4 °C.
2. Pre-warm pre-digestion buffer and both tubes of equilibration buffer at +37 °C.
3. Attach the base of the gentleMACS Perfuser 2 with the lid-grid-clamp assembly onto the gentleMACS Perfusion Sleeve on the gentleMACS Octo Dissociator with Heaters. Ensure that the adjuster is in the correct position.
4. Place a Heating Unit onto the gentleMACS Perfuser 2.

   ▲ **Note:** Do not attach the base without the lid to the instrument when installing the Heating Unit. This may lead to malfunction of the Heating Unit.
5. Carefully transfer 8 mL of warm pre-digestion buffer into the gentleMACS Perfuser 2 by using one of the two luer openings.
6. Start the gentleMACS Program **37C_m_HPK_1.**

Step	Duration	Buffer required
Priming	5 seconds	Pre-digestion buffer
Initial perfusion	28 seconds	Pre-digestion buffer
Washing	2×28 seconds 1×12 minutes	Pre-digestion buffer
Equilibration	30 seconds	Equilibration buffer
Enzymatic perfusion	15 minutes	Enzyme digestion mix

Table 2: Overview of gentleMACS Program steps.

▲ **Note:** After each step the program will pause to allow the exchange of buffers. Do not click **Resume** in the pop-up window until the buffer has been exchanged. Buffer exchange is done manually using disposable glass Pasteur pipettes with elongated tips. To aspirate buffer, insert the pipette tip through one of the luer lock connectors to the base bottom. It is recommended to connect the pipette to a liquid suction pump for more convenience.

▲ **Note:** Do not exchange buffers after priming. The program will automatically pause. Press **Resume** only after the tissue sample has been placed in the gentleMACS Perfuser 2 (see step 16).

7. Remove the assembly of lid-clamp-grid from the base and place it in a petri dish.
8. Cut an approx. 15 cm piece from the surgical thread and form a loop with a diameter that is slightly larger than the heart.
9. Sacrifice the animal and open the chest. Fix the costal arches with pins on the preparation table. Locate the pericardium, a thin whitish membrane that surrounds the heart, and gently remove it with tweezers to expose the heart.
10. Place the prepared loop over the heart and use inverted curved tweezers to push the thread loop behind the atria. Use tweezers to gently lift the heart and close the ligation by pulling the thread at its ends until the knot is tight. Remove the excess thread. Resect the ligated heart by cutting the tissue behind the ligation.

    ▲ **Note:** Make sure that the heart as well as the ligation are not damaged during heart dissection.
11. Place the heart in the middle of the grid with the ventricles side-by-side each showing to one of the two grid extensions.

    ▲ **Note:** Left and right ventricles normally have slightly different reddish colors.
12. Push the clamp completely down to fix the heart between grid and clamp.
13. Transfer the assembly of lid-clamp-grid back onto the base of the gentleMACS Perfuser 2.
14. Turn the lid complex slowly counterclockwise on the base until the arrow on the lid (▼) aligns with the arrow on the base (▼). At this position, the lid complex drops to a lower position.

    ▲ **Note:** If necessary, the lid can slightly be pushed down.
15. Turn the lid clockwise until the arrow on the lid (▼) aligns with the arrow on the base (▲) to secure the gentleMACS Perfuser 2.
16. Resume the program that remained paused after priming to continue with the initial perfusion step. Attention: After resuming the program, immediately turn the adjuster by rotating it counterclockwise by 360°.
17. After the initial perfusion step, the program pauses to start the washing phase. This phase consists of three cycles of buffer exchange. The program will pause after each cycle. When the program is paused, remove the used pre-digestion buffer manually with a disposable glass Pasteur pipette with an elongated tip and add 8 mL of fresh pre-digestion buffer. Resume the program after each pause.
18. After the last washing step, remove the pre-digestion buffer and add 8 mL equilibration buffer.
19. Resume the program.
20. During the equilibration step, prepare the enzyme digestion mix for one perfusion by adding 200 µL Enzyme D, 65 µL Enzyme T, and 10 µL Enzyme A to 6 mL pre-warmed equilibration buffer.
21. After the equilibration step, remove the equilibration buffer and add 6 mL of enzyme digestion mix.
22. Resume the program.

    ▲ **Note:** The program ends after the enzymatic perfusion step. Do not discard the used enzyme digestion mix, it will be used in step 25.

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23. Detach Heating Unit and gentleMACS Perfuser 2 from the gentleMACS Instrument.
24. Unscrew the lid and transfer the assembly of lid-clamp-grid into a petri dish.
25. Pour the used enzyme digestion mix from the base into a gentleMACS C Tube. Discard the base.
26. Push up the clamp using tweezers. Transfer the tissue from the grid into a petri dish.
27. Discard the lid with the attached clamp-grid assembly.
28. Remove the thread from the heart and transfer the heart into the C Tube containing the used enzyme digestion mix.
29. Tightly close the C Tube and attach it upside down onto a regular gentleMACS Sleeve of the gentleMACS Octo Dissociator with Heaters.
30. Run the gentleMACS Program **HPK_CR_1**.

    ▲ **Note:** The program consists of incubation steps for most of the time without rotation.

    ▲ **Note:** Dissociation of the perfused heart with manual methods might decrease yield and viability of the isolated cells.
31. Put a 15 mL tube on ice and place a MACS SmartStrainer (100 µm) on it.
32. After termination of the program, detach the C Tube from the gentleMACS Octo Dissociator with Heaters.
33. Open the C Tube and transfer the cell suspension onto the MACS SmartStrainer (100 µm).

    ▲ **Note:** Avoid producing air bubbles.
34. Wash the C Tube with 4 mL of stop buffer. Transfer this onto the MACS SmartStrainer (100 µm).
35. Centrifuge the cell suspension at 140×g for 1 minute at +4 °C. Remove supernatant completely. The supernatant can be used for further enrichment of non-parenchymal cells.
36. Loosen the cell pellet by tapping two conical tubes against each other several times.
37. Resuspend the cells in an appropriate buffer for downstream applications, for example, 5 mL of stop buffer. Mix by inverting several times.
38. Keep the cell suspension on ice and immediately proceed to downstream applications.

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