Miltenyi Biotec

Liver Perfusion Kit
mouse and rat

Order no. 130-128-030

Contents

1. Description

This product is for research use only.

Components	6 vials, containing: **1 vial of Enzyme D (lyophilized powder)** **1 vial of Enzyme R (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)** **100 mL Buffer P (20×)** **1 mL Reagent C** **1 mL Reagent E**
Capacity	For 25 digestions. The specified number of digestions is valid for digesting of up to 1.2 g of liver tissue per perfusion following the protocol in chapter 2.2.
Storage	Upon arrival store the enzymes at 2–8 °C, and all other components at room temperature. Reconstitute the enzymes before the date indicated on the box label. For information about reconstitution and storage after enzyme reconstitution refer to chapter 2.1.

1.1 Principle of the Liver Perfusion Kit

By using the gentleMACS™ Perfusion Technology rodent liver 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. Rodent liver tissues are enzymatically digested using the components of the Liver Perfusion Kit, mouse and rat which loosen 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 Liver Perfusion Kit, mouse and rat has been developed for the gentle, rapid, and efficient generation of single-cell suspensions from rodent liver in combination with the gentleMACS Perfusion Technology. It is optimized for a high yield of parenchymal rodent liver cells, i.e., hepatocytes, while preserving most cell surface epitopes. Dissociated cells can subsequently be cultured or non-parenchymal cells can be isolated using MACS Technology. Furthermore, the single-cell suspensions can be phenotyped and other functional, genetic, or proteomic studies can be performed.

1.3 Applications

- Dissociation of rodent liver for subsequent functional assays
- Cultivation of hepatocytes
- Phenotyping or enumeration of cells by flow cytometry or fluorescence microscopy
- Cell isolation using MACS Technology

1.4 Reagent and instrument requirements

- PBS buffer: Phosphate-buffered saline (PBS), pH 7.2
- DMEM with stable glutamine and 5% FCS
- MACS SmartStrainers (100 µm) (50 filters, # 130-098-463; 4×25 filters, # 130-110-917)
- Disposable glass Pasteur pipettes with elongated tip (230 mm)
- gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (25 tubes, # 130-093-237; 4×25 tubes, # 130-096-334)
- gentleMACS Perfusers (# 130-128-151)
- gentleMACS Perfusion Sleeves (# 130-128-752)
- Water bath
- Liquid suction pump
- Cooling centrifuge with a swinging bucket rotor
- Petri dishes (diameter: 6 cm and 10 cm)
- 15 and 50 mL reagent tubes
- Surgical instrument set
- (Optional) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) Debris Removal Solution (# 130-109-398)
- (Optional) 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).

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2. Protocol

The perfusion process takes place in the gentleMACS Perfuser. The gentleMACS Perfuser consists of different components (Figure 1) that need to be handled at different steps of the protocol.

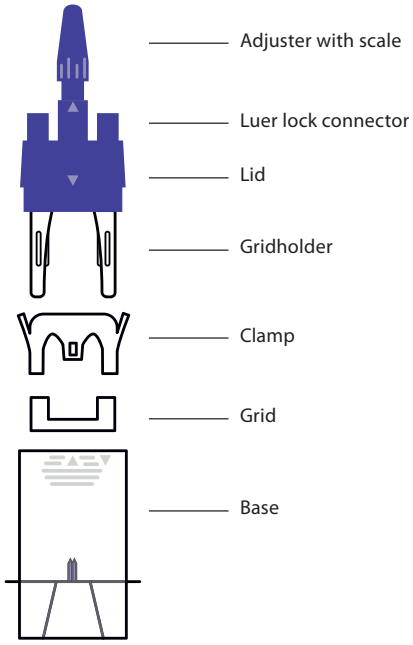

Figure 1: Components of the gentleMACS Perfuser.

▲ For details on the use of the gentleMACS Octo Dissociator with Heaters, the gentleMACS Perfusers, and the Perfusion Sleeves refer to the gentleMACS Dissociator user manual or respective data sheets.

▲ Make sure that the gentleMACS Octo Dissociator with Heaters runs with the latest software update. The perfusion programs are included in the Miltenyi program folder from software version H22 onwards. The latest software update can be requested under https://www.miltenyibiotec.com/lp/2018/gentlemacs-software-update.html.

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

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

2.1 Reagent preparation

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

▲ Buffer P (20×) should be stored at room temperature and pre-warmed at 37 °C for at least 45 minutes before first use. Shake the buffer thoroughly before use.

Preparation of reconstitution buffer

Prepare reconstitution buffer by diluting 400 µL Buffer P (20×) with 7.6 mL sterile water. Add 16 µL Reagent C and mix well.

▲ **Note:** Precipitation of Buffer P (20×) might occur at 4 °C. In this case, pre-warm Buffer P (20×) at 37 °C for about 45 minutes to dissolve precipitate completely.

Enzyme reconstitution

1. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL reconstitution buffer. Invert the vial after closing the lid and wait 5–10 minutes to dissolve the powder. Do not pipette up and down to dissolve the enzyme. 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 immediately before taking out the required reaction volume.

2. Prepare Enzyme R by reconstitution of the lyophilized powder in the vial with 3 mL reconstitution buffer. Invert the vial after closing the lid and wait 5–10 minutes to dissolve the powder. Do not pipette up and down to dissolve the enzyme. 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 immediately before taking out the required reaction volume.

3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL of reconstitution buffer. 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 immediately before taking out the required reaction volume.

Preparation of perfusion buffers

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

1. Prepare 62 mL Buffer P (1×) by diluting 3.1 mL Buffer P (20×) with 58.9 mL sterile, distilled water. Buffer P (1×) is used as the basis to prepare the perfusion buffers.
2. Prepare the perfusion buffers according to the table below.

Table 1: Preparation of perfusion buffers.

Perfusion buffers	Buffer P (1×)	Reagent C
Pre-digestion buffer	44 mL	–
Equilibration buffer	18 mL	36 µL

3. Take 9 mL of the prepared equilibration buffer and transfer to a separate tube. This will be used to prepare the enzyme digestion mix. Enzymes will be added freshly shortly before the enzyme digestion mix is used (chapter 2.2, step 18).

2.2 Liver perfusion

▲ A maximum of 1.2 g of liver tissue can be perfused in one gentleMACS Perfuser.

▲ The time from the dissection of the liver to the start of the perfusion should not exceed 10 minutes (step 9). Longer time spans will impact cell yield and viability. If this time interval cannot be maintained, the liver tissue can be stored up to 2 hours in cold MACS Tissue Storage Solution supplemented with Reagent E (1:200) on ice.

▲ If working with mouse, it is recommended to use the biggest mouse liver lobe (left lateral lobe) instead of the whole mouse liver.

▲ If working with rat, it is recommended to use one of the small lobes (e.g. the dorsal right lobe). Alternatively, use a liver piece between 10 and 15 mm in diameter that does not exceed 1.2 g of total weight.

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▲ The adjuster of the lid of the gentleMACS Perfuser is already provided in the correct position (original position) for the perfusion of mouse liver. **Do not turn the adjuster if mouse liver shall be perfused!**

▲ If using rat liver or other samples with similar height, turn the adjuster by rotating it clockwise by 360° (one complete turn). The needles will penetrate deeper into the tissue.

▲ If the position of the adjuster has been altered by accident, it can be re-adjusted to the original position by turning it counterclockwise to the highest position and then slightly back until the ".5" on the adjuster's scale matches the arrow head on the adjuster. Be careful not to accidently turn the adjuster once the mouse liver is placed in the gentleMACS Perfuser, as needles might pierce through the liver which will hamper the perfusion.

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

1. Pre-heat water bath at 37 °C.
2. Pre-warm pre-digestion buffer and both tubes of equilibration buffer at 37 °C.
3. Attach the base of the gentleMACS Perfuser with the lid onto the gentleMACS Perfusion Sleeve on the gentleMACS Octo Dissociator with Heaters. Leave out the grid and the clamp.

   ▲ **Note:** Ensure the adjuster is in the correct position according to the tissue to be perfused.
4. Place a Heating Unit onto the gentleMACS Perfuser.

   ▲ **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 by using one of the two luer openings.
6. Start the appropriate gentleMACS Program for mouse **37C_m_LIPK_1** or for rat **37C_r_LIPK_1.** The table below describes the overview of steps and duration for each program.

Table 2: Overview of the gentleMACS Programs steps.

Step	Duration	Buffer required
Priming	5 seconds	Pre-digestion buffer
Initial perfusion	30 seconds	Pre-digestion buffer
Washing	3×30 seconds 1×13 minutes	Pre-digestion buffer
Equilibration	30 seconds	Equilibration buffer
Enzymatic perfusion	**37C_m_LIPK_1**: 10 minutes **37C_r_LIPK_1**: 12.5 minutes	Enzyme digestion mix

   ▲ **Note:** After each step the programs 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 tip. 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:** After priming, there is no need for buffer exchange.
7. After the priming step, the program will automatically pause and will be resumed later after the tissue sample was placed (see step 15).
8. Put the grid into a 10 cm petri dish.
9. Sacrifice the animal and transfer liver into a petri dish. Carefully dissect an appropriate liver lobe and rinse with PBS buffer.

   ▲ **Note:** Make sure that the liver lobe is not injured during dissection.

   ▲ **Note:** If the time interval between dissection of the liver and start of the perfusion exceeds 10 minutes, the tissue can be stored in cold MACS Tissue Storage Solution supplemented with Reagent E (1:200) on ice.
10. Place the liver lobe in the center of the grid.
11. Squeeze the clamp and attach it to the grid by pushing it down until the clamp touches the surface of the petri dish to fix liver between grid and clamp.

    ▲ **Note:** Make sure that the two channels of the grid used for buffer removal are not blocked by wrong assembly of grid and clamp.
12. Remove the lid of the gentleMACS Perfuser and connect it by pushing the gridholder through the clamp into the grid containing the tissue.

    ▲ **Note:** A click will emerge at each side of the gridholder if the gridholder has been properly connected to the grid.
13. Transfer the assembly of lid-clamp-grid back onto the base of the gentleMACS Perfuser.
14. Turn the lid slowly counterclockwise without pressure on the base until the lid drops to a lower position. Then, screw the thread of the lid clockwise without tilting to tightly close the gentleMACS Perfuser.
15. Resume the program that remains paused after priming to continue with the initial perfusion step.

    ▲ **Note:** No change of buffer is needed at this step since the buffer inside the gentleMACS Perfuser was only used for priming the system.
16. After the initial perfusion step, the program will pause to start the washing phase. This phase consists of 4 cycles of buffer exchange, and the program will pause after each cycle. During the pause, remove the used pre-digestion buffer manually with a disposable glass pasture pipette with an elongated tip, and then add 8 mL of fresh pre-digestion buffer. Resume the program only after the buffer has been exchanged.
17. After the last washing step, remove the pre-digestion buffer and add 8 mL equilibration buffer. Resume the program only after the buffer has been exchanged.
18. Prepare the enzyme digestion mix for one perfusion during the equilibration phase. Add 110 µL Enzyme D, 110 µL Enzyme R, and 30 µL Enzyme A to 9 mL warm equilibration buffer.
19. After the equilibration step, remove the equilibration buffer and add 8 mL enzyme digestion mix. Resume program only after buffer has been exchanged.
20. After the enzymatic perfusion step, the program ends. Do not discard the used enzyme digestion mix which will be used in step 23.
21. Detach the Heating Unit and gentleMACS Perfuser from the gentleMACS Instrument.
22. Unscrew the lid and transfer the lid-clamp-grid assembly into a 6 cm petri dish.
23. Pour the used enzyme digestion mix from the base into a gentleMACS C Tube and discard the base.
24. Push up the clamp using tweezers and transfer the tissue from the grid into the C Tube containing the used enzyme digestion mix. Discard the lid with attached clamp-grid assembly.

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25. Tightly close the C Tube and attach it upside down onto a regular gentleMACS Sleeve of the gentleMACS Dissociator.

▲ **Note:** Ensure that the regular gentleMACS Sleeve is used for C Tubes.

26. Run the gentleMACS Program **LIPK_HR_1.**

▲ **Note:** Disruption of the perfused liver with manual methods might decrease the yield and viability of the isolated cells.

▲ **Note:** If a significant amount (>30%) of undissociated liver is still attached to the rotor of the C Tube after the dissociation step, open the C Tube and transfer the undissociated liver tissue back into the liquid. Close the C Tube and run the gentleMACS Program for another 30 seconds.

27. Put a 15 mL tube on ice and place a MACS SmartStrainer (100 µm) on it.

28. After termination of the program, detach the C Tube from the gentleMACS Dissociator.

29. Open the gentleMACS Tube and transfer the cell suspension onto the MACS SmartStrainer (100 µm).

▲ **Note:** Avoid producing air bubbles.

30. Wash the C Tube with 6 mL of DMEM with glutamine and 5% FCS. Transfer this onto the MACS SmartStrainer (100 µm).

31. Centrifuge the cell suspension at 30×g for 5 minutes at 4 °C. Remove supernatant completely.

▲ **Note:** The supernatant can be used for further enrichment of non-parenchymal cells.

▲ **Note:** To increase the purity of hepatocytes, a density gradient centrifugation (see step 2.3) can be performed.

32. Carefully resuspend the cell pellet containing hepatocytes by slowly pipetting up and down in an appropriate buffer for downstream application, for example, with 5 mL cold PBS or PEB buffer.

▲ **Note:** Do not resuspend by vortexing.

33. (Optional) For further enrichment of hepatocytes, proceed to 2.3.

2.3 (Optional) Density gradient centrifugation using Debris Removal Solution

If the purity or viability of the obtained hepatocytes is not sufficient for downstram applications, viable hepatocytes can be further enriched by using a density gradient centrifugation employing the Debris Removal Solution.

▲ Volumes given below are for the cell suspension obtained after perfusion of up to 1.2 g liver tissue (one perfusion) as starting material.

▲ Always use buffers and solutions pre-cooled at 4 °C.

1. Prepare the debris removal solution mix in a 15 mL tube according to the table below.

Table 3: Preparation of the debris removal solution mix.

	Debris removal solution mix	
	For mouse liver	For rat liver
Debris Removal Solution	3 mL	2.3 mL
Cold 1× PBS buffer	3 mL	3.6 mL

2. Carefully underlay 5 mL of cell suspension (from step 32) with the debris removal solution mixture which has the higher density. To avoid mixing the phases slowly dispense the debris removal mixture with a glass Pasteur pipette at the tube bottom by gravity flow.

3. Centrifuge at 500×g for 10 minutes at 4 °C with full acceleration and full brake. Aspirate supernatant completely and discard it.

4. Carefully resuspend cell pellet with 5 mL of cold medium or appropriate buffer for downstream applications.

▲ **Note:** Do not resuspend by vortexing.

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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