Miltenyi Biotec

Umbilical Cord Dissociation Kit
human

Order no. 130-105-737

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 B (lyophilized powder)** **1 vial of Enzyme A (lyophilized powder)** **1 vial of 2.5 mL Enzyme P** **2 vials of 30 mL Buffer L**
Size	For 25 digestions. The specified number of digestions is valid when digesting umbilical cord pieces that do not exceed 0.5 g in weight following the protocols in chapter 2.2.
Storage	Upon arrival immediately store Enzyme P in aliquots at –20 °C. Store all other components at 2–8 °C upon arrival. 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 Umbilical Cord Dissociation Kit

Human umbilical cord can be dissociated to single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins which maintain the structural integrity of tissues.
In a first step, the umbilical cord is soaked with enzymes which degrade the extracellular matrix. In a second step, single-cells are freed from the extracellular matrix by using the gentleMACS™ Dissociators. After dissociation remaining particles are removed by filtration using MACS® SmartStrainer.
Cells should be processed immediately for downstream applications, such as cell culture, cell separation, or cell analysis.

1.2 Background information

The Umbilical Cord Dissociation Kit, human enables the gentle and efficient generation of single-cell suspensions from human umbilical cord. The kit is not certified as GMP product and has been particularly developed to enable a quality control of frozen umbilical cord samples for umbilical cord tissue banks by detecting mesenchymal stem cells (MSCs) directly after dissociation by flow cytometry without long-standing cultivation of the cells. Additionally, the cells can be also used in downstream applications such as cell culture or molecular studies.

1.3 Applications

- Dissociation of fresh or frozen human umbilical cord pieces for subsequent cell separations using MACS Technology.
- Cultivation of umbilical cord cell populations, e.g., mesenchymal stem cells or endothelial cells.
- Phenotyping or enumeration of umbilical cord cell populations by flow cytometry or fluorescence microscopy.

1.4 Reagent and instrument requirements

- PBS: phosphate-buffered saline pH 7.2
- DMEM (# 130-091-437)
- MACS SmartStrainers (100 µm) (# 130-098-463)
- 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) 70 % ethanol
- (Optional) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.
- (Optional for expansion of MSCs) StemMACS™ MSC Expansion Media Kit XF, human (# 130-104-182) or StemMACS MSC Expansion Media, human (# 130-091-680)
- (Optional for differentiation of MSCs) StemMACS AdipoDiff Media, human (# 130-091-677), StemMACS ChondroDiff Media, human (# 130-091-679), or StemMACS OsteoDiff Media, human (# 130-091-678)

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2. Protocols

▲ 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 preparation

1. Prepare aliquots of appropriate volume of Enzyme P to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months.
2. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL Buffer L supplied with the kit. Do not try to resuspend by pipetting or vortexing. Invert vial after closing and wait 5–10 minutes 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. 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 Buffer L 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.
4. Prepare Enzyme B by reconstitution of the lyophilized powder in the vial with 1 mL Buffer L 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. For cell culture experiments subsequent to tissue dissociation, Enzyme B should be sterile filtered prior to aliquoting.

2.2 Umbilical cord dissociation protocol

2.2.1 Dissociation of frozen tissue

1. Cryopreservation:
   Wash umbilical cord in an appropriate buffer. Cut umbilical cord into small pieces (<5 mm diameter) to allow quick penetration of cryopreservation buffer. Add a cryoconservation medium containing 10% DMSO and transfer sample in a cryopreservation tube or bag. Freeze sample slowly at –70 °C, e.g., by using an appropriate styrofoam box. After 24 hours transfer sample into a liquid nitrogen tank.
2. Thawing of samples:
   Switch on a water bath at 37 °C. Thaw the sample (about 0.5 g of tissue) completely using the water bath. Transfer the sample into a tube containing 45 mL of DMEM. Invert the tube for 1 minute and transfer the sample onto a MACS SmartStrainer (100 µm).
3. Add 2.2 mL of Buffer L into the gentleMACS C Tube.
4. Transfer the retained sample from the strainer into the C Tube.
5. Add 100 µL of Enzyme D, 62.5 µL of Enzyme P, 4 µL of Enzyme B, and 10 µL of Enzyme A into the C Tube.

   ▲ **Note:** Enzyme A, B, and D can be premixed before addition into the C Tube. Do not premix any of the enzymes with Enzyme P.
6. Tightly close C Tube beyond the first resistance.
   If using the heating function of the gentleMACS Octo Dissociator with Heaters, attach the C Tube upside down onto the sleeves of the gentleMACS Octo Dissociator with Heaters. It has to be ensured that the sample material is still located in the area of the rotor/stator before starting the gentleMACS program. Run the gentleMACS Program **37C_h_UCDK_1** and continue with step 10.
7. Incubate sample in a water bath at 37 °C for 3 hours.
8. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.

   ▲ **Note:** It has to be ensured that the sample material is still located in the area of the rotor/stator before starting the gentleMACS program.
9. Run the gentleMACS program **h_cord_01**.
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Perform a short centrifugation step to collect the sample material at the tube bottom.
12. Apply sample to a MACS SmartStrainer (100 µm).

    ▲ **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.
13. Wash the filter with 5 mL of DMEM.

    ▲ **Note:** For maximum cell recovery, rinse the used C Tube with wash buffer before transfer to the filter.
14. Discard the filter, invert tube after closing and centrifuge sample at 300×g for 15 minutes at room temperature.
15. Aspirate supernatant completely and resuspend cells with medium or an appropriate buffer to the required volume for further applications.
16. Process cells immediately for further applications.

    ▲ **Note:** MSCs, endothelial cells, and leukocytes can be distinguished after dissociation by the surface markers CD90, CD73, CD34, and CD45 (MSCs are $CD90^+CD73^+CD34^-CD45^-$; endothelial cells are $CD34^+CD73^+CD45^-CD90^-$). If using the MSC Phenotyping Kit, be aware that CD105 is degraded during the dissociation step and that endothelial cells cannot be distinguished from leukocytes by this kit.

    ▲ **Note:** For expansion of MSCs we recommend using the StemMACS MSC Expansion Media Kit XF, human (# 130-104-182).

2.2.2 Dissociation of fresh tissue

1. Wash umbilical cord in an appropriate buffer or cell culture medium, e.g., MACS Tissue Storage Solution or DMEM.

   ▲ **Note:** For subsequent cell culture soak umbilical cord in 70 % ethanol for 30 seconds, then cut off the ends, and wash at least once in an appropriate buffer. Remove as much blood as possible.
2. Add Buffer L (volume according to the table in step 5) into the gentleMACS C Tube.
3. Cut off a piece of the umbilical cord (weight according to the table in step 5) and transfer it into the C Tube.
4. Cut the tissue in small pieces of 2–4 mm using scissors which reach the bottom of the C Tube.

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5. Add enzymes into the C Tube according to the following table:

Tissue [g]	Buffer L [mL]	Enzyme D [µL]	Enzyme P [µL]	Enzyme B [µL]	Enzyme A [µL]
0.5	2.2	100	62.5	4	10
1	4.4	200	125	8	20
2–4	8.8	400	250	16	40

▲ **Note:** Enzyme A, B, and D can be premixed before addition into the C Tube. Do not premix any of the enzymes with Enzyme P.

6. Tightly close C Tube beyond the first resistance.
If using the heating function of the gentleMACS Octo Dissociator with Heaters, attach the C Tube upside down onto the sleeves of the gentleMACS Octo Dissociator with Heaters. Run the gentleMACS Program **37C_h_UCDK_1** and continue with step 10.
7. Incubate sample in a water bath at 37 °C for 3 hours.
8. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator.
▲ **Note:** It has to be ensured that the sample material is still located in the area of the rotor/stator before starting the gentleMACS program.
9. Run the gentleMACS program **h_cord_01**.
10. After termination of the program, detach C Tube from the gentleMACS Dissociator.
11. Perform a short centrifugation step to collect the sample material at the tube bottom.
12. Apply sample to a MACS SmartStrainer (100 µm).
▲ **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.
13. Wash the filter with DMEM. Use twice as much DMEM as volume in tube.
▲ **Note:** For maximum cell recovery, rinse the used C Tube with wash buffer before transfer to the filter.
14. Discard the filter, invert tube after closing and centrifuge sample at 300×g for 15 minutes at room temperature.
15. Aspirate supernatant completely and resuspend cells with medium or an appropriate buffer to the required volume for further applications.
16. Process cells immediately for further applications.
▲ **Note:** MSCs, endothelial cells, and leukocytes can be distinguished after dissociation by the surface markers CD90, CD73, CD34, and CD45 (MSCs are $CD90^+CD73^+CD34^-CD45^-$; endothelial cells are $CD34^+CD73^+CD45^-CD90^-$). If using the MSC Phenotyping Kit, be aware that CD105 is degraded during the dissociation step and that endothelial cells cannot be distinguished from leukocytes by this kit.
▲ **Note:** For expansion of MSCs we recommend using the StemMACS MSC Expansion Media Kit XF, human (# 130-104-182).

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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