Miltenyi Biotec

Whole Skin Dissociation Kit
human

Order no. 130-101-540

Contents

1. **Description**
   - 1.1 **Principle of the Whole Skin Dissociation Kit**
   - 1.2 **Background information**
   - 1.3 **Applications**
   - 1.4 **Reagent and instrument requirements**
2. **Protocol**
   - 2.1 **Reagent preparation**
   - 2.2 **Whole skin dissociation protocol**
3. **Appendix**

1. Description

Components	4 vials, containing: **2.5 mL of Enzyme P** **1 vial Enzyme A (lyophilized powder)** **1 vial Enzyme D (lyophilized powder)** **30 mL of Buffer L**
Size	For 50 digestions. The specified number of digestions is valid for 4 mm biopsies following the protocol in chapter 2.2.
Storage	Uppon arrival immediately store Enzyme P aliquoted at –20 °C. Store all other components at 2–8 °C upon arrival. The expiration date is 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 Whole Skin Dissociation Kit

Human whole skin can be dissociated into single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins, which maintain the structural integrity of tissues.
Dermis is not separated from epidermis. In a first step, the human skin 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™ Dissociator.
Cells should be processed immediately for downstream applications, such as cell separation, cell culture, cellular or molecular analyses.

1.2 Background information

The Whole Skin Dissociation Kit, human enables the gentle and efficient generation of single-cell suspensions from human skin tissue. The kit has been particularly developed for the isolation of fibroblasts from diverse human skin biopsies. Furthermore, dissociated cells can be subsequently cultured or isolated using MACS® Technology.

1.3 Applications

- Dissociation of human skin tissue for the cultivation of fibroblasts.
- Phenotyping or enumeration of human skin cell populations by flow cytometry.

1.4 Reagent and instrument requirements

- Cell culture medium, e.g., DMEM with stable glutamine (# 130-091-438) with 10% fetal bovine serum (FBS), 10 mM HEPES, 1% Penicillin/Streptavidin, 1mM sodium pyruvat, and 1× non-essential amino acids.
- Pre-Separation Filters, 70 µm (# 130-095-823)
- gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) Tool for taking punch biopsies (e.g., Biopsy Punch)
- (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.

▲ The protocol has been optimized for the digestion of adult human skin from breast or abdominal reduction surgery.

▲ Up to three punch biopsies (4 mm each) can be used per digestion. When working with bigger punch biopsies, cut the biopsy into pieces with a maximum diameter of 4 mm.

2.1 Reagent preparation

1. Prepare aliquots of appropriate volume of Enzyme P to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C.
2. Prepare Enzyme D by reconstitution of the lyophilized powder in the vial with 3 mL Buffer L supplied with the kit. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C.
3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL Buffer L supplied with the kit. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C.

2.2 Whole skin dissociation protocol

1. Wash human skin tissue sample in an appropriate buffer or cell culture medium, e.g., MACS Tissue Storage Solution.
2. Carefully scrape off the subcutaneous fat using a scalpel. If the diameter of the skin sample exceeds 4 mm in diameter, take one or more 4 mm diameter punches by rotating down the tool (e.g., Biopsy Punch) through epidermis and dermis. Store punches in an appropriate buffer or cell culture medium, e.g., MACS Tissue Storage Solution, until needed.
3. Transfer 435 µL of Buffer L and 12.5 µL of Enzyme P into the gentleMACS C Tube and mix carefully.

   ▲ **Note:** Some epitopes (e.g. CD4 and CD8) are sensitive for Enzyme P. If these epitopes are to be remained in the single-cell suspension, omit the addition of Enzyme P which lowers cell yields (refer to table in chapter 3.).
4. Add 50 µL of Enzyme D and 2.5 µL of Enzyme A into the C Tube and mix carefully (keep buffer at the bottom of the tube).

   ▲ **Note:** Enzyme A and Enzyme D can be premixed before addition into the C Tube. Do not premix Enzyme P with Enzyme A or Enzyme D.
5. Transfer one sample of skin tissue (4 mm) into the C Tube containing the enzyme mix and tightly close it.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.

   ▲ **Note:** Up to 3 samples (4 mm each) can be processed per C Tube if an overnight incubation is chosen in step 6.
6. Incubate sample in a water bath at 37 °C for 3 hours or overnight.

   ▲ **Note:** Longer incubation time increases cell yield.
7. After incubation dilute the sample by adding 0.5 mL of cold cell culture medium.
8. 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.
9. Run the gentleMACS Program **h_skin_01**.

   ▲ **Note:** The extracellular matrix is not completely dissociated after the dissociation step but do not repeat the step as it reduces cell yields.
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 the cell suspension to a Pre-Separation Filter, 70 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.
13. Wash the filter with 4 mL of cold cell culture medium.

    ▲ **Note:** (Optional) To collect remaining cells in the C Tube add the cold medium first to the C Tube and then on top of the filter.
14. Discard the filter and centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
15. 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 separation or flow cytometry.
16. Process cells immediately for further applications.

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3. Appendix

Target cell yield per sample

The following table shows the dependence of target cell yield per 4 mm punch biopsy of adult abdominal skin based on incubation length and addition of Enzyme P. The yields vary depending on donor and skin locus.

	3 hours incubation	Overnight incubation
with Enzyme P	120,000 total cells 40,000 $CD90^+$ 10,000 $CD3^+$ 4,000 $CD1c^+$	380,000 total cells 80,000 $CD90^+$ 20,000 $CD3^+$ 6,000 $CD1c^+$
without Enzyme P	45,000 total cells 10,000 $CD90^+$ 3,500 $CD3^+$ 1,500 $CD1c^+$	110,000 total cells 22,000 $CD90^+$ 8,000 $CD3^+$ 3,000 $CD1c^+$

Typical staining

Epitopes which are intact after the dissociation procedure are, for example, CD90 (fibroblasts), CD45 (leukocytes), CD3 (T cells), and CD1c (dendritic cells).
Epitopes which are degraded after the dissociation procedure are, for example, CD4 and CD8. The degradation can be avoided by omitting Enzyme P but yields will be decreased.

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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