Miltenyi Biotec

Epidermis Dissociation Kit ACF
human

Order no. 130-103-464

Contents

1. Description

Components	3 vials, containing **1 vial of Enzyme G, ACF (lyophilized powder)** **2.5 mL of Enzyme P, ACF** **1 vial of Enzyme A, ACF (lyophilized powder)**
Size	For 100 digestions. The specified number of digestions is valid for 4 mm biopsies following the protocol in chapter 2.2.
Storage	Upon arrival immediately store Enzyme P, ACF 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 Epidermis Dissociation Kit ACF

Human epidermal tissue can be dissociated to single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins, which maintains the structural integrity of tissues.
In a first step, the epidermal cell layer is removed from the dermal cell layer after enzymatic treatment over night at 4 °C. The epidermal tissue is then further digested enzymatically and dissociated into a single-cell suspension by using the gentleMACS Dissociators. Cells should be processed immediately for downstream applications, such as cell separation, cellular, or molecular analyses.

1.2 Background information

The Epidermis Dissociation Kit ACF, human enables the gentle and efficient generation of single-cell suspensions from human epidermal tissue. It has been particularly developed for the isolation of keratinocytes from diverse human skin biopsies. Furthermore, dissociated cells can be subsequently cultured or isolated using MACS® Technology.
This product is animal component-free (ACF) as it does not contain any primary raw materials derived directly from animals during the manufacturing process.

1.3 Applications

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

1.4 Reagent and instrument requirements

- RPMI 1640
- PBS: phosphate-buffered saline pH 7.2
- PB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA) by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with PBS. Keep buffer cold (2–8 °C).
- Pre-Separation Filter (70 µm) (# 130-095-823)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 4 °C
- gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (25 tubes, # 130-093-237; 4×25 tubes, # 130-096-334)
- (Optional) MACS Tissue Storage Solution (# 130-100-008)
- (Optional) Tool for taking punch biopsies (e.g., Biopsy Punch)

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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.

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

▲ Up to five 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

▲ Prepare Enzyme G, ACF by reconstitution of the lyophilized powder in the vial with 3 mL of sterile, distilled water. Do not vortex. Produce aliquots of appropriate volume. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

▲ Prepare aliquots of appropriate volume of Enzyme P, ACF. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

▲ Prepare Enzyme A, ACF by reconstitution of the lyophilized powder in the vial with 0.25 mL of sterile, distilled water. Do not vortex. Produce aliquots of appropriate volume. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

2.2 Epidermis dissociation protocol

2.2.1 Separation of epidermis from dermis (day l)

1. Wash human skin tissue in an appropriate buffer or cell culture medium, e.g., MACS Tissue Storage Solution.
2. Remove subcutaneous fat using scissors. 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 1 mL of RPMI 1640 and 25 µL of Enzyme G, ACF to into a 2 mL tube and mix carefully. Keep on ice.
4. Transfer up to five samples of skin tissue (4 mm diameter) into the 2 mL tube.
5. Incubate sample for 14–18 hours at 4 °C using the MACSmix Tube Rotator (12 rpm).

2.2.2 Automated dissociation of epidermis using the gentleMACS Dissociator (day 2)

1. Prewarm the water bath to 37 °C.
2. Take the biopsy out of the 2 mL tube and peel off the epidermis from the dermis using curved tweezers.
3. (Optional) Transfer the epidermis without dermis back to the 2 mL tube and incubate for additional 2 hours at 37 °C.

   ▲ **Note:** This step will increase yield of Langerhans cells.
4. Prepare enzyme mix by adding 1 mL of RPMI 1640, 25 µL of Enzyme P, ACF, and 1 µL of Enzyme A, ACF into a new 2 mL tube. Keep on ice.

   ▲ **Note:** Do not mix Enzyme P, ACF and Enzyme A, ACF directly.
5. Transfer the epidermis into the 2 mL tube containing the enzyme mix.
6. Incubate for 60 minutes at 37 °C in a water bath.

   ▲ **Note:** It has to be ensured that the sample material is located in the enzyme mix during the incubation time.
7. Lay the epidermis directly on the rotator/stator of the gentleMACS C Tube. Transfer the remaining liquid of the 2 mL tube in the C Tube.

   ▲ **Note:** It has to be ensured that the sample material is located in the enzyme mix in the area of the rotor/stator before starting the gentleMACS Program as the epidermis might easily stick to the tube wall and won´t be dissociated.
8. Stop enzymatic reaction by adding 1 mL of cold PB buffer.
9. 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.
10. Run the gentleMACS Program **B**.
11. After termination of the program, detach C Tube from the gentleMACS Dissociator.
12. Perform a short centrifugation step to collect the sample material at the tube bottom.
13. Resuspend sample by pipetting up and down and apply the cell suspension to a Pre-Separation Filter (70 µm) placed on a 15 mL tube.
14. Wash the Pre-Separation Filter (70 µm) with 1 mL of cold PB Buffer.

    ▲ **Note:** (Optional) To collect remaining cells in the C Tube add buffer first to the C Tube and then on top of the filter.
15. Discard the Pre-Separation Filter (70 µm) and centrifuge sample at 300×g for 10 minutes at room temperature. Aspirate supernatant completely
16. Resuspend cells by pipetting up and down with PB buffer or an appropriate buffer to the required volume for further applications.

    ▲ **Note:** If cell clumps occur after the washing step, add another 1 µL of Enzyme A, ACF per mL of cell suspension. Mix gently and incubate for 5 minutes at 37 °C in a water bath. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely and repeat step 16.
17. Process cells immediately for further applications.

3. Appendix

Target cell yield per sample

Typical target cell yields per 4 mm punch biopsy of adult abdominal skin are about 8×10^4 total cells (mostly keratinocytes) and 3×10^3 Langerhans cells.

Typical staining

Epitopes which are intact after the dissociation procedure are, e.g., CD29 and CD49f (both positive for keratinocytes), CD45, CD207, CD1a, and HLA-DR (all positive for Langerhans cells).
Epitopes which are degraded after the dissociation procedure are, for example, CD117 (marker for melanocytes).

140-004-332.04

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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