Miltenyi Biotec

Epidermis Dissociation Kit

mouse

Order no. 130-095-928

Contents

1. Description

Components	4 vials, containing **13 mL of Buffer S (20× Stock Solution)** **1 vial of Enzyme G (lyophilized powder)** **2.5 mL of Enzyme P** **1 vial of Enzyme A (lyophilized powder)**
Size	For 25 digestions. The specified number of digestions is valid when digesting skin with an average weight of 1.5 g following the protocol 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 Enzymes G and A 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

Epidermal tissue 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 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 either using the gentleMACS™ Dissociators or by manual handling.
Cells should be processed immediately for downstream applications, such as cell separation, cellular or molecular analysis.

1.2 Background information

The Epidermis Dissociation Kit, mouse enables the gentle and efficient generation of single-cell suspensions from mouse epidermal tissue. The kit has been particulary developed for the isolation of epidermal Langerhans cells.

1.3 Applications

- Dissociation of mouse skin tissue for the isolation of Langerhans cells using the Epidermal Langerhans Cell MicroBead Kit (# 130-095-408).
- Phenotyping or enumeration of epidermal cell populations by flow cytometry.

1.4 Reagent and instrument requirements

- 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).
- MACS SmartStrainers, 70 µm (# 130-098-462)
- (Optional) gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937)
- (Optional) gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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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.

▲ The protocol is developed for the digestion of skin derived from 6–10 weeks old female C57 BL/6 or Balb/c mice.

▲ **Note:** It is not recommended to use skin derived from younger or older mice.

▲ An amount of up to 1.5 g skin tissue can be used per digestion, which corresponds approximately to the weight of back skin derived from two mice. When working with less than 1.5 g use the same volumes as indicated below.

2.1 Reagent and instrument preparation

▲ Prepare 1× Buffer S by adding, for example, 1 mL of 20× Buffer S aseptically to 19 mL of sterile, distilled water. Store at 2–8 °C.

▲ **Note:** Handle under sterile conditions.

▲ Prepare Enzyme G 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.

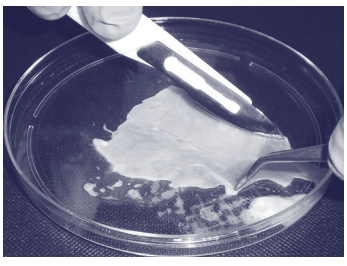
▲ Prepare aliquots of appropriate volume of Enzyme P. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

▲ Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL 1× Buffer S. Do not vortex. Produce aliquots of appropriate volume. Store aliquots at –20 °C. Avoid repeated freeze-thaw-cycles.

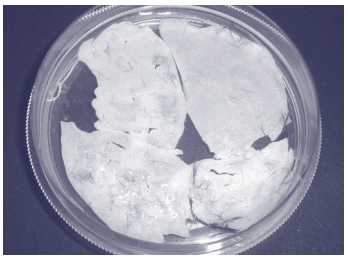
2.2 Epidermis dissociation protocol

2.2.1 Separation of epidermis from dermis (day l)

1. Remove hair completely from the back of the mice by plucking against the growth direction of the hair.
2. Cut skin at the base of the tail with a transverse cut using scissors and incise then upwards on both sides. Peel the tissue off with tweezers and make a transverse cut at the neck.
3. Wash tissue by placing the skin in a 100 mm petri dish containing 20 mL of PBS buffer.
4. Determine weight of the tissue to make sure that the limit of 1.5 g per digestion is not exceeded.
5. Transfer tissue to a new 100 mm petri dish with the epidermal side facing downwards.
6. Carefully scrape off the subcutaneous fat using a scalpel.



7. Subdivide skin of one mouse in 2 pieces.
8. Prepare enzyme mix by adding 100 µL of Enzyme G to 3.9 of mL PBS buffer in a 50 mm petri dish. Mix well.
9. Place tissue pieces on top of the enzyme mix with the dermal side facing downwards.

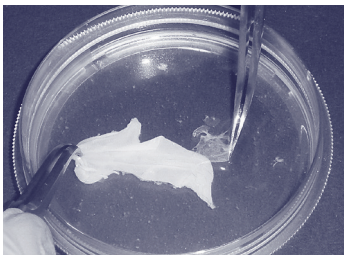


10. Incubate at 4 °C for 16 hours.

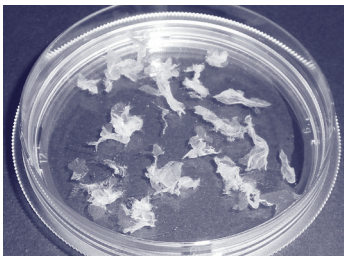
▲ **Note:** Do not extend incubation time!

2.2.2 Dissociation of epidermis (day 2)

1. Prewarm the water bath to 37 °C.
2. Peel off the epidermis from the dermis using curved tweezers.



3. Transfer the epidermis into a new 50 mm petri dish containing 4 mL of 1× Buffer S and cut into smaller pieces.



4. For manual dissociation proceed with chapter 2.2.2.1.

   For automatic dissociation using the gentleMACS Dissociator proceed with chapter 2.2.2.2.

2.2.2.1 Manual dissociation

1. Prepare enzyme mix by adding 3.9 mL of 1× Buffer S, 100 µL of Enzyme P, and 20 µL of Enzyme A into a 50 mL tube.
2. Transfer epidermis pieces into the tube containing the enzyme mix. The sample material must not stick to the wall of the tube.
3. Incubate for 20 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.
4. Stop enzymatic reaction by adding 4 mL of PB buffer.
5. Pour sample onto a MACS SmartStrainer, 70 µm, placed on a 50 mL tube. Pass sample through the 70 µm mesh by using a plunger of a 1 mL syringe.

   ▲ **Note:** It is not recommended to use a pipette tip to add the sample to the MACS SmartStrainer, 70 µm, as the tissue tends to stick to the pipette tip wall.

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6. Wash the MACS SmartStrainer, 70 µm, with 5 mL of PB buffer and discard it.
7. Repeat steps 5 and 6 using a new MACS SmartStrainer, 70 µm.
8. Centrifuge sample at 300×g for 10 minutes at room temperature.
9. Aspirate supernatant completely and resuspend cells with PB buffer or an adequate buffer to the required volume for further applications.

   ▲ **Note:** Do not vortex.

   ▲ **Note:** If cell clumps occur after the washing step, add another 10 µL of Enzyme A 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 and repeat step 9.
10. Process cells immediately for further applications.

2.2.2.2 Automated dissociation using the gentleMACS™ Dissociator

1. Prepare enzyme mix by adding 3.9 mL of 1× Buffer S, 100 µL of Enzyme P, and 20 µL of Enzyme A into a gentleMACS™ C Tube.
2. Transfer epidermis pieces into the gentleMACS C Tube containing the enzyme mix. The sample material must not stick to the wall of the tube.
3. Incubate for 20 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.
4. Stop enzymatic reaction by adding 4 mL of PB buffer.
5. 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 enzyme mix in the area of the rotor/stator before starting the gentleMACS Program.
6. Run the gentleMACS Program **B**.
7. After termination of the program, detach C Tube from the gentleMACS Dissociator.
8. Apply sample to a MACS SmartStrainer, 70 µm, placed on a 50 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.
9. Wash the MACS SmartStrainer, 70 µm, with 10 mL of PB Buffer.
10. Discard the MACS SmartStrainer, 70 µm, and centrifuge sample at 300×g for 10 minutes at room temperature.
11. Aspirate supernatant completely and resuspend cells with PB buffer or an adequate buffer to the required volume for further applications.

    ▲ **Note:** Do not vortex.

    ▲ **Note:** If cell clumps occur after the washing step, add another 10 µL of Enzyme A 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 and repeat step 11.
12. Process cells immediately for further applications.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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