Dec 01, 2025

Isolation of Single-Cell Suspensions from Full-Thickness Human Skin

DOI
https://dx.doi.org/10.17504/protocols.io.kxygx4jkkl8j/v1

Liang Luo[1], Da-Hai Hu[1]

[1]Department of Burns and Cutaneous Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, Shanxi, China, 710032

HFSCA-A single-cell lan...

clayluo

DOI: https://dx.doi.org/10.17504/protocols.io.kxygx4jkkl8j/v1

Protocol Citation: Liang Luo, Da-Hai Hu 2025. Isolation of Single-Cell Suspensions from Full-Thickness Human Skin. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx4jkkl8j/v1

Protocol status: Working
We use this protocol and it's working

Created: December 01, 2025

Last Modified: December 01, 2025

Protocol Integer ID: 233856

Keywords: cell suspension, separate processing of epidermis, thickness human skin this protocol, cell application, thickness human skin, human skin, cell viability, epidermis, scrna, cell, rna quality, dermi

Funders Acknowledgements:
Liang Luo
Grant ID: 82372524

Disclaimer

This protocol is intended for research purposes only. The authors and publishers are not responsible for any misuse or damage resulting from the application of this protocol.

Abstract

This protocol describes the preparation of single-cell suspensions from full-thickness human skin and separate processing of epidermis and dermis for downstream single-cell applications (for example, scRNA-seq). It contains recommendations to improve RNA quality, cell viability, and reproducibility while keeping the original workflow and reagents as close as possible to the submitted protocol.

Materials

- Fresh full-thickness human skin (1 × 2 cm recommended; process immediately)
- Dispase II (stock prepared in PBS without Ca/Mg)
- Collagenase I, II, IV (stock concentrations and activity indicated on manufacturers' vials)
- Hyaluronidase (Sigma H3884)
- DNase I (10 mg/mL)
- Trypsin/EDTA 0.25% (optional for epidermis) or Accutase/neutral protease as gentler alternative
- DMEM/F12 with 10% heat-inactivated FBS
- RNase inhibitor (e.g., RNaseOUT or Enzymatics RNase inhibitor)
- Cell strainers: 100 µm, 70 µm
- Cell counter and viability dye (Trypan Blue or AO/PI)
- 15 mL and 50 mL RNase-free Falcon tubes
- Cold centrifuge capable of 4°C and 300 x g
- Rotator or orbital shaker at 37°C

Reagents (working concentrations)

1. **Epidermis digestion (per 1 mL):**
   - 0.25% Trypsin/EDTA, 37°C, 20–30 min OR Accutase for 20–30 min

2. **Dermis digestion:**
   - Collagenase I: final 2 mg/mL
   - Collagenase II: final 0.5–1 mg/mL
   - Collagenase IV: final 0.5 mg/mL
   - Hyaluronidase: final 0.1–0.2 mg/mL
   - DNase I: final 50–200 µg/mL (0.05–0.2 mg/mL) to reduce viscosity from free DNA

3. **Hypodermis digestion**
   - **Digestion buffer (per 100 mL):** HBSS (with Ca2+/Mg2+) or DMEM + 0.5–1% BSA (w/v). Warm to 37°C before use. Add Collagenase to final 1.0 mg/mL immediately before use. Optionally add DNase I to 50–100 µg/mL.
   - **ACK RBC lysis buffer:** 150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA (store at 4°C).

Equipment

- 37°C water bath or shaking incubator
- Ice bucket and refrigerated centrifuge set to 4°C
- Microscope for counting and QC
- Cell counter (e.g., Countess)
- Optional: GentleMACS dissociator or equivalent mechanical dissociation device

Before start

Timing note: timing given for ~1 g skin processed in small tubes; scale up proportionally. Perform all steps on ice where indicated and use RNase-free consumables.

Procedure

1 Tissue collection and transport (10–30 min). Collect full-thickness human skin (1 × 2 cm) fresh and immediately place into ice-cold RNase-free PBS (no Ca2+/Mg2+). Keep samples on ice and process as soon as possible (within 1–2 h preferred).

2 Trim and weigh (5–10 min). Transfer subcutaneous fat with sterile scissors and forceps; record tissue weight. Cut tissue into 2–3 mm wide strips for Dispase treatment. If tissue is very thick, trim the deep reticular dermis to improve enzyme penetration.

3 Epidermis–dermis separation with Dispase II (30–45 min). Prepare Dispase II working solution (e.g., final 2.0–2.4 U/mL in PBS without Ca/Mg). Place up to ~0.1 g tissue per 1 mL Dispase solution (do not overload; recommended tissue:enzyme volume ratio ≤ 1:3 w/v). Incubate at 37°C for 30–40 min (gentle rocking). Using sterile forceps, peel the epidermis from dermis carefully; proceed immediately to separate digestion for each fraction.

4 Epidermis digestion and processing (20–35 min). Place epidermal pieces into 1 mL 0.25% trypsin (or Accutase) at 37°C for 20–30 min with gentle agitation. Monitor until epidermis becomes translucent.
Neutralize with equal volume DMEM/F12 + 10% FBS.
Filter through 100 µm then 70 µm strainers into 15 mL tubes.
Centrifuge 300 × g, 4°C for 5 min. Aspirate supernatant and resuspend in cold PBS.
Count cells with Trypan Blue; target viability ≥ 80–90% for scRNA-seq.

5 Dermis digestion and processing (60–90 min). Cut dermal tissue into ~1 mm^3 pieces and place into 1 mL digestion mix (see Reagents: Option A or B).
Incubate at 37°C with shaking (200–300 rpm) for 40–60 min. For Option B, 60 min is typical. Check progress every 15–20 min.
Mechanically triturate gently (e.g., 10–30 gentle pipette strokes) to release single cells.
Filter through 70 µm strainer, wash filter with 5–10 volumes PBS to recover cells.
Centrifuge 500 × g, 4°C for 5–10 min, aspirate and resuspend in cold PBS with 0.04% BSA for counting.
Add DNase I (final 50–200 µg/mL) during digestion or wash steps if high viscosity observed.

6 Hypodermis / SVF isolation (adipose) (40–90 min). Rinse adipose 2–3× with cold sterile HBSS to remove blood/debris; mince to ~1–2 mm^3 if not already fragmented. Keep tissue cold and record weight.
Place minced tissue on coarse mesh (~300 µm) and wash with cold HBSS (~200–300 mL) to remove free lipids and debris. Remove visible clots.
Transfer tissue to tube; add pre-warmed digestion buffer with Collagenase (~1.0 mg/mL), using ~2–3 mL buffer per gram tissue. Loosen cap for gas exchange.

Incubate at 37°C with gentle agitation (~100 rpm) for 40–80 min. Check every 10–15 min; endpoint when suspension is uniformly cloudy and few fragments remain. Optionally add DNase I (50–100 µg/mL).
Pass digest through 300 µm then 70 µm filters; rinse filters with HBSS + 0.5% BSA.
Centrifuge filtered suspension at ~150–200 × g, 4°C, 3–5 min to float adipocytes.
Carefully aspirate infranatant (SVF) avoiding lipid layer.
Centrifuge infranatant at ~300 × g, 4°C, 5–10 min to pellet SVF. Discard supernatant.
If RBC contamination present, resuspend pellet in ACK lysis buffer at room temperature for 1–5 min, dilute with excess HBSS/PBS and centrifuge at 300 × g for 5 min at 4°C.
Wash once with DMEM/F12 + 10% FBS (optional) or HBSS, centrifuge 300 × g, 5 min.
Wash again with sterile PBS (Ca2+/Mg2+ free) 300 × g, 5 min.
Resuspend in appropriate buffer (e.g., 0.04% BSA in PBS) and count viable cells.

7 Washing and QC (10–15 min).
Wash cell pellet twice with cold PBS (300 × g, 4°C, 5 min).
Resuspend in appropriate buffer for downstream application (e.g., 0.04% BSA in PBS for 10x).
Measure cell concentration and viability. Aim for 700–1200 cells/µL for 10x Chromium loading depending on desired recovered cell number.
If RBC contamination is present, perform an RBC lysis step (short incubation with RBC lysis buffer, then wash).

8 Short-term holding (4°C).
Keep live single-cell suspensions on ice or at 4°C for short term (ideally process immediately for downstream assays).

Timing

9 Total hands-on time: ~2–3 h per sample (1 g tissue) depending on digestion choices and downstream QC.

Troubleshooting

10 Low viability (<70%).
Reduce digestion time, use gentler enzymes (Accutase), work quickly at 4°C after digestion and avoid Triton/permeabilization; minimize mechanical shear.

11 Low cell yield.
Ensure adequate mincing (small fragments), increase enzyme concentration slightly or incubation time by 10–20 min; do not overload digestion volume.

12 High debris/aggregation.
Include DNase I during digestion; filter with 40–70 µm strainers; perform a density gradient cleanup if needed.

13 Poor RNA quality.
Process fresh where possible; use RNase-free reagents and add RNase inhibitor where compatible; minimize time at room temperature.

Anticipated results

14 From 1 g of healthy human full-thickness skin expect ~0.5–5 million cells depending on tissue source, processing efficiency and patient factors. Viability for scRNA-seq should be >80% (ideally >90%). Filtered single-cell suspension should be free of large aggregates and have minimal RBC contamination.

Minimal edits / corrections made to original protocol

15 Clarified working concentrations and units for Dispase II, collagenases, hyaluronidase and DNase.
Standardized centrifugation speeds and times and specified 4°C where appropriate.
Replaced ambiguous volume/units (e.g., '200 ml DPBS' corrected to '200 µL' where appropriate in counting steps).
Added RNase-free handling notes and recommended RNase inhibitors where compatible.

Protocol references

1. Emont, M. P., Jacobs, C., Essene, A. L., Pant, D., Tenen, D., Colleluori, G., Rosen, E. D. (2022). A single-cell atlas of human and mouse white adipose tissue. Nature, 603, 926–933. https://doi.org/10.1038/s41586-022-04518-2
2. Philippeos, C., Telerman, S. B., 6 Watt, F. M. et al. (2018). Spatial and single-cell transcriptional profiling identifies functionally distinct human dermal fibroblast subpopulations. Journal of Investigative Dermatology, 138(4), 811–825. https://doi.org/10.1016/j.jid.2018.01.016
3. Ścieżyńska A, Nogowska A, Sikorska M, et al. Isolation and culture of human primary keratinocytes—a methods review. Experimental Dermatology. 2019;28(2):107-112. doi:10.1111/exd.13860
4. Reynolds, G., Çataltepe, Ö., et al. (2021). Developmental cell programs are co-opted in inflammatory skin disease. Science, 371, eaba6500. https://doi.org/10.1126/science.aba6500

Acknowledgements

The authors would like to thank the staff of the Department of Burns and Cutaneous Surgery at Xijing Hospital for their support and assistance throughout this study.