

Mar 20, 2024

Tissue Procurement SOP - Discarded Human Skin

DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlko7bwv5r/v1>

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Protocol Citation: Alberto Pappalardo, Rolando Perez-Lorenzo, Angela Christiano 2024. Tissue Procurement SOP - Discarded Human Skin. [protocols.io https://dx.doi.org/10.17504/protocols.io.j8nlko7bwv5r/v1](https://dx.doi.org/10.17504/protocols.io.j8nlko7bwv5r/v1)

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Protocol status: Working

We use this protocol and it's working

Created: March 06, 2024



Last Modified: March 20, 2024

Protocol Integer ID: 96197

Keywords: tissue procurement sop, handling of human skin tissue, discarded human skin, tissue procurement for the project, tissue procurement, human skin tissue, human skin from human subject, human skin the purpose, tissue, sop, procedure, instructions to clinician, document, proper sample storage, human subject

Funders Acknowledgements:

NCI

Grant ID: 5UG3CA275686

Abstract

The purpose of this procedure is to provide instructions to clinicians, researchers and support staff for the collection and handling of human skin tissue. Specifically, this document describes the proper sample storage, delivery, de-identification, processing, and data collection of discarded human skin from human subjects during tissue procurement for the project 5UG3CA275686.

Materials

16% paraformaldehyde (Electron Microscopy Sciences #15710)

Dulbecco's Phosphate Buffered Saline (Gibco #14190-144)

Sucrose powder (Millipore Sigma #S0389)

ddH₂O

Ethanol 200 proof (Decon labs #2701)

Liquid Nitrogen

O.C.T. compound (Fisher healthcare #4585)

Surgical grade tweezers, scissors, and scalpel blades

Tissue Source

1 **Discarded neonatal foreskin:**

The neonatal human foreskin is a leftover of circumcision surgeries, which would otherwise be discarded.

Discarded adult skin:

The adult human skin is a leftover of reconstructive surgeries, which would otherwise be discarded.

Sample processing:

2 **Samle collection**

- 2.1 1. Following excision, the samples are promptly stored in biospecimen containers on ice, deidentified, and labelled with a number (e.g. #1) which will be referred to for registering and transmitting the specimen information.

a. The clinical staff is responsible for collecting and transmitting to the research team the following information: age, sex, race, ethnicity, body site provenance (e.g. abdominal), and health status of the donor.

b. The research staff will annotate the Fitzpatrick phototype of the skin specimen.

2. As soon as the specimen is available, the clinical staff notifies by email the research team, which will immediately pick up the specimen (bring an ice-filled styrofoam box to pick up the specimen). If the specimen is not picked up within 30 minutes, annotate the time.

3. At the laboratory, the specimen is immediately re-labelled and processed.

2.2 **a. Labelling:**

i. The alphanumeric ID indicates the project (SEN), the year (e.g. 2023) of samples acquisition, age group (neonatal or adults) and sequential number of acquisition (1,2,3,...) such as SEN23A1 is the first adult sample acquired in 2023 for the SenNet project.

ii. Register in the specimens spreadsheet (stored in the Christiano lab google drive) the specimen ID, recording the date of acquisition, anatomical location, and donor's age, sex, race, ethnicity, and health status if known.

2.3 **b. Cell Isolation.** Follow standard skin cells isolation protocol for each cell type.



2.4 c. Tissue Banking.

i. Fresh frozen:

1. Insert tissue aliquot in a 50 ml tube
2. Freeze the tube in liquid nitrogen (5 minutes) then move to -80C freezer.

ii. Fixed frozen OCT-embedded:

1. Immerse the tissue aliquot (max 2 cm x 2 cm x 1 cm) in a 50 ml tube with 4% paraformaldehyde (Electron Microscopy Sciences #15710) diluted in DPBS. Use a ratio tissue/fixative of 1:10 and store at 4C for 16 hours.
2. Discard the fixative, rinse with DPBS.
3. Wash twice for 5' by filling the tube with DPBS.
4. Fill the tube with 35% sucrose solution and keep 24h at 4C.
5. Embed in O.C.T. compound.
6. Freeze the block by placing on top of metal cassettes immersed in liquid nitrogen in a closed Styrofoam box (the block does not touch directly the liquid nitrogen)
7. After 10' move to -80C freezer.

iii. Fixed paraffin-embedded:

1. Immerse the tissue aliquot (max 2 cm x 2 cm x 1 cm) in a 50 ml tube with 4% paraformaldehyde (Electron Microscopy Sciences #15710) diluted in DPBS. Use a ratio tissue/fixative of 1:10 and store at 4C for 16 hours.
2. Discard the fixative, rinse with H₂O.
3. Wash twice for 60' by filling the tube with H₂O.
4. Replace H₂O with 50% ethanol and incubate 3 hours
5. Replace with 70% ethanol and proceed with standard embedding protocol or store in 70% ethanol at 4C.