



Montreal Neurological Institute and Hospital
McGill University

CBIG-02-012

SKIN BIOPSY (SAMPLING PROCEDURE, TRANSPORT AND CULTURE)

Version:	1.0	Supersedes:	NA
Category:	Procedural	Effective date:	2-Oct-2020

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1. REVISION HISTORY

Version	Summary of revisions	Effective Date
1.0	Initial	2-Oct-2020

2. SCOPE AND APPLICATION

This protocol is to be used for the *in vitro* sampling, transport and culture of a skin biopsy.

3. REFERENCE TO OTHER SOP OR DOCUMENTS

When adopting this SOP for local use, please reference *C-BIG Repository: CBIG-02-012 Skin Biopsy (Sampling Procedure, Transport and Culture)*.

3.1 Reference to Other C-BIG SOPs or Documents

1. C-BIG Repository: CBIG-02-013 Fibroblast Culture
2. C-BIG Repository: CBIG-03-002 RPM Conversion

3.2 Reference to External SOPs or Documents

1. Axe Neurosciences, Centre de recherche du CHU de Québec, Protocol : Procédure de prélèvement et de transport pour une biopsie de peau (immortalisation cellulaire)
2. Axe Neurosciences, Centre de recherche du CHU de Québec, Protocol : Culture primaire de fibroblaste à partir d'une biopsie de peau (immortalisation cellulaire)

4. PERSONNEL QUALIFICATION AND RESPONSIBILITIES

To be read by all personnel who process a skin biopsy. All personnel who read this SOP should sign the form found in the reading log binder.

5. ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
C-BIG	Clinical Biological Imaging and Genetic Repository
CO ₂	Carbon Dioxide
DMEM	Dulbecco's Modified Eagle's Medium
FBS	Fetal Bovine Serum
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
PBS	Phosphate Buffer Saline
PenStrep	Penicillin Streptomycin
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
°C	Degree Celsius

6. MATERIALS AND EQUIPMENT

The materials and equipment listed below are recommendations only and may be substituted by alternative/equivalent products more suitable for site-specific task or procedure.

NOTE: All disposable items are sterile (gamma-irradiated) unless otherwise specified. All equipment, disposables or reagents can be substituted with equivalent materials following evaluations and approval, unless specified otherwise.

NOTE: All sample contact materials must be suitable for RNA work (i.e., RNase-free). Use clean gloves at all times to prevent inadvertent RNase contamination during processing.

Material/Equipment	Material/Equipment (site specific)
Phosphate buffered saline (1X), pH 7.4, CA ²⁺ and Mg ²⁺ free	Wisent; Cat # 311-010-CL
DMEM	Thermofisher; Cat # 11995-073
PenStrep	Thermofisher; Cat # 15140-122
FBS	Thermofisher; Cat # 12484028
Amphotericine	Sigma; Cat # A2942
Polypropylene conical tubes, 15 mL	
Centrifuge with swinging bucket rotor	Eppendorf centrifuge 5810, Cat # 022625101; with rotor S-4-104, Cat # 5820759003
Disposable serological pipets 5 mL	
Disposable serological pipets, 10 mL	

Rack for 15 mL tube	
Forceps	
Scissors	
Scalpel	
Petri Dish (60 X 15 mm)	Fisher; Cat # 08-772B
Incubator	
Pipet-Aid	
Class II biological safety cabinet	

7. PROCEDURES

PRECAUTIONS: All biological samples derived from human source are considered to be biohazardous. Use appropriate precautions when working with such samples (i.e. personal protection equipment such as gloves, lab coat and safety glasses). All waste (samples and related contact materials) must be placed in marked biohazardous waste containers and disposed of under hospital guidelines.

7.1 Skin Biopsy (sampling procedure)

1. Prepare transport medium:

445 mL DMEM
50 mL FBS
5 mL PenStrep

2. Prepare a 15 mL polypropylene sterile conical tube containing 10 mL of transport medium and a proper label with the identification of the sample.

3. Take skin biopsy in aseptic conditions and put the biopsy immediately in the tube containing transport medium.

NOTE: The skin biopsy needs to be around 2X2 mm.

7.2 Skin biopsy (transport)

1. Put the conical tube on a metal rack inside the transport container.
2. Send the package to the recipient.

NOTE: The package and the biopsy must be kept at room temperature.

NOTE: The biopsy must be delivered in maximum three (3) hours post-surgery.

7.3 Skin biopsy (culture)

1. Prepare wash solution and fibroblast medium:

Wash Solution:	Fibroblast Medium:
495 mL of PBS 1X	445 mL DMEM
5 mL of PenStrep	50 mL FBS
	5 mL PenStrep
	2.5 mg/mL Amphotericine

2. Remove the transport medium and add 10 mL of washing solution, lightly shake the conical tube.
3. Centrifuge for 5 min at 500Xg using a centrifuge with swinging buckets.
4. Remove supernatant and add 10 mL of washing solution, then shake lightly.
5. Centrifuge for 5 min at 500Xg using a centrifuge with swinging buckets.
6. Remove supernatant and add 10 mL of washing solution.
7. Transfer all the contents of conical tube into a petri dish (biopsy and washing solution).

NOTE: Additional washing step can be added if a lot of red blood cells are present. The red blood cells can inhibit fibroblast development.

8. With sterile forceps and scissors, prepare the biopsy by removing any fat present.
9. In the centre of a new petri dish, with a scalpel, draw at the bottom a small grid equivalent to the surface of the biopsy.
10. Put the biopsy on the grid.
11. Incubate under the hood for at least 15 min with the petri dish slightly open, so the biopsy can adhere to the dish.

NOTE: The biopsy can easily come off the the petri dish. Use extreme caution for the following steps.

12. Add drop by drop 2 to 3 mL of fibroblast medium.
13. Incubate at 37°C (5% CO₂).

14. After 24 to 72 hours, replace the fibroblast medium with 2 to 3 mL of new medium.
15. Change the fibroblast medium every two (2) days (normally Monday, Wednesday and Friday).

NOTE: Cells should start to come out of the biopsy five (5) to seven (7) days after it was placed in culture. When the confluency reaches between 50 to 75 percent, continue with SOP *CBIG-02-013 Fibroblast Culture*.

NOTE: The skin biopsy can be re-used by being put in a new petri dish with a grid (follow section 7.3 steps 10 to 15). By changing the medium regularly, new fibroblast will come out of the biopsy. This passing can be done several times on the same skin biopsy until the skin biopsy does not adhere anymore.

8. QUALITY CONTROL / QUALITY ASSURANCE

All the equipment used should be monitored, cleaned and calibrated as by their specific SOPs.

Reagents with an expiry date should be monitored and used before this date. If used after expiry date, it should be recorded.

9. APPENDICES/FORMS

9.1 Appendix A – Sample processing form: Skin Biopsy