Montreal Neurological Institute and Hospital
McGill University

CBIG-02-013

FIBROBLAST CULTURE

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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* culture of fibroblasts.

3. REFERENCE TO OTHER SOP OR DOCUMENTS

When adopting this SOP for local use, please reference *C-BIG Repository: CBIG-02-013 Fibroblasts Culture*.

3.1 Reference to Other C-BIG SOPs or Documents

1. C-BIG Repository: CBIG-03-002 RPM Conversion
2. C-BIG Repository: CBIG-03-001 Different Methods to Count Cells

3.2 Reference to External SOPs or Documents

1. 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
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
FBS	Fetal Bovine Serum
mg	Milligram
min	Minute
mL	Milliliter
PBS	Phosphate Buffer Saline
PenStrep	Penicillin Streptomycin
QA	Quality Assurance
QC	Quality Control
RPM	Rotation Per Minute
RT	Room Temperature
SOP	Standard Operating Procedure
°C	Degree Celsius
%	Percent

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 substitutes 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). Always use clean gloves 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	
Polypropylene conical tubes, 50 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	

Disposable serological pipets, 25 mL	
TrypleExpress	Thermofisher; Cat # 12604021
Forceps	
T75 flask	Fisher; Cat # 1368065
T175 flask	Fisher; Cat # 1012613
Pipet tips, 200 µL	
Micropipets 100-1000 µL	
DMSO	Fisher Scientific; Cat # D1391
Cryovials round-bottom, 1.8 mL	Nunc Biobanking and Cell Culture Cryogenic Tubes, Thermo Scientific; Cat # 368632
Nalgene Cryo 1°C "Mr. Frosty" Freezing Container	Nalgene Nunc; Cat # 5100-0001
2-propanol	
-80°C Freezer	
Liquid Nitrogen Tank	
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 to be considered 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 Fibroblast culture

1. When fibroblasts reach a confluency of 80 to 90% in ¾ of the petri dish, remove the biopsy and the medium. The confluency is verified with a microscope.

NOTE: The biopsy can be reused, follow the SOP *CBIG-02-012 Skin Biopsy (Sampling Procedure, Transport and Culture).*

2. Add 2 mL of PBS.
3. Remove the PBS and add 1 mL of TrypleExpress.
4. Incubate one (1) to three (3) minutes at 37°C or until all fibroblast are detach from the petri dish. After the incubation, a slight tap on the side will help in the complete detachment of the fibroblasts. The fibroblasts detachment can be confirmed with a microscope.
5. Add 2 mL of fibroblast medium in the petri dish, pipet up and down to mix well.

Fibroblast Medium:
445 mL DMEM
50 mL FBS
5 mL PenStrep
2.5 mg/mL Amphotericine

6. Transfer all the fibroblasts into a 15 mL conical tube.

7. Count fibroblast, please refer to SOP *CBIG-03-001 Different Methods to Count Cells.*
8. Transfer all into a T75 flasks and add 10 mL of fibroblast medium.

NOTE: This is considered passage zero (0).

9. Change fibroblast medium every two (2) days (for simplicity every Monday, Wednesday and Friday) until a confluency between 80 and 90% is reached.
10. Remove the medium and add 10 mL of PBS.
11. Remove the PBS and add 3 mL of TrypleExpress.
12. Incubate one (1) to three (3) minutes at 37°C. After the incubation, a slight tap on the side will help in the complete detachment of the fibroblasts. The fibroblasts detachment can be confirmed with a microscope.
13. Add 7 mL of fibroblast medium into the flask, pipet up and down to mix well.
14. Count fibroblast, please refer to SOP *CBIG-03-001 Different Methods to Count Cells.*
15. Split cells between freezing (see section 7.2 Cryopreservation of fibroblasts) or passing.

NOTE: Around one (1) million cells is needed per T175 flask.

16. Centrifuge for 5 min at 500Xg.

17. Remove supernatant and add 5 mL of fibroblast medium.
18. Transfer cells into a T175 flask (passage of one (1)) and add 20 mL of fibroblast medium.
19. Change fibroblast medium every two (2) days (for simplicity every Monday, Wednesday and Friday) until cells are 80 to 90 % confluent.
20. Remove medium and add 10 mL of PBS.
21. Remove PBS and add 5 mL of TrypleExpress.
22. Incubate one (1) to three (3) minutes at 37°C. After the incubation, a slight tap on the side will help in the complete detachment of the fibroblasts. The fibroblasts detachment can be confirmed with a microscope.
23. Add 8 mL of fibroblast medium into the flask.
24. Count cells, please refer to SOP *CBIG-03-001 Different Methods to Count Cells.*
25. Split cells between freezing (see section 7.2 Cryopreservation of fibroblasts) or passing.

NOTE: If passing the cells, please redo the steps 16 to 25 of section 7.1. At least one (1) million cells are needed per T175 flask. Cell passing can be done until enough cells have been cryopreserved.

7.2 Cryopreservation of fibroblasts.

1. Put the fibroblasts you wish to freeze in a new 50 mL conical tube.
2. Fill the fibroblast suspension up to 50 mL with PBS and mix.
3. Centrifuge at RT for 5 min at 500Xg using a centrifuge with swinging buckets.
4. Discard the supernatant and keep the cell pellet.
5. Re-suspend cells in X mL of freezing medium where X corresponds to the number of vials to prepare.

NOTE: The freezing medium is composed of 90% cold FBS and 10% of DMSO.

NOTE: A concentration of $1X10^6$ cells/mL per vial is ideal.

6. Immediately following the addition of the freezing medium, aliquot fibroblast into one (1) mL aliquots in labelled cryovials and place in Mr. Frosty freezing container (equilibrated to RT).
7. Immediately transfer Mr. Frosty to a -80°C freezer for a minimum of 48 hours and maximum of seven (7) days and then transfer to a liquid nitrogen tank in the vapor phase for long term storage.

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: Fibroblast Culture

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