Montreal Neurological Institute and Hospital
McGill University

CBIG-02-010

THAWING OF CRYOPRESERVED CELLS

Version:	1.0	Supersedes:	NA
Category:	Procedural	Effective date:	18-Jun-2020

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1. REVISION HISTORY

Version	Summary of revisions	Effective Date
1.0	Initial	18-Jun-20

2. SCOPE AND APPLICATION

CBIG has a vast collection of frozen material that can be used for various purposes. CBIG employees must be able to thaw viable cells in a consistent and reproducible way. This SOP describes the procedure to thaw cells in a controlled manner followed by the processes of your choice based on your purposes.

3. REFERENCE TO OTHER SOP OR DOCUMENTS

When adopting this SOP for local use, please reference C-BIG Repository: CBIG-02-010 Thawing of Cryopreserved Cells

3.1 Reference to Other C-BIG SOPs or Documents

1. C-BIG Repository: CBIG-03-001 Different methods to count cells
2. C-BIG Repository: CBIG-03-002 RPM conversion

3.2 Reference to External SOPs or Documents

1. Experimental Therapeutics Program: ETP-P-0006 Thawing of Cryopreserved Cells

4. PERSONNEL QUALIFICATION AND RESPONSIBILITIES

To be read by all personnel who wish to thaw cells from the repository or external sources. All personnel who read this SOP should sign the form found in the training log binder.

5. ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
BSC	Biological Safety Cabinet
C-BIG	Clinical Biological Imaging and Genetics Repository
EtOH	Ethanol
HI HuAB Serum	Heat Inactivated Human AB Serum
Min	Minutes
mL	Milliliter
PPE	Personal Protective Equipment
PBS	Phosphate Buffered Saline
QA	Quality Assurance
QC	Quality Control
RT	Room Temperature
SOP	Standard Operating Procedure
µL	Microliter
˚C	Degrees 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 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). Use clean gloves at all times to prevent inadvertent RNAse contamination during processing.

Material/Equipment	Material/Equipment (site specific)
Centrifuge with swinging bucket rotor	Eppendorf centrifuge 5810, Cat # 022625004; with rotor S-4-104, Cat # 5820759003
Water Bath	
Disposable serological pipets, 5 mL	
Disposable serological pipets, 10 mL	
Sterile polypropylene conical tubes 15 mL	
Sterile polypropylene conical tubes 50 mL	
70% EtOH	
Appropriate culture media of your choice	
Dry Ice	
PBS	Wisent; Cat # 311-010-CL

Transfer pipets	
Incubator (5% CO2)	
Microscope	
BSC	

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.

PRECAUTIONS: Wear appropriate PPE equipment when handling cryovials stored in liquid nitrogen tank (lab coat, gloves and face shield). There is always a risk of exploding cryovials (a result of liquid nitrogen leaking into the vials and expanding rapidly upon thawing).

NOTE: All manipulations to be performed under aseptic conditions.

7.1 Thawing Process

1. Prepare the appropriate medium of your choice by pre-warming it in a water bath at 37°C.

2. Retrieve a cryovial containing the frozen cells from its storage area (-80˚C or a liquid nitrogen tank) and place on either ice or dry ice based on availability to minimize thawing during transportation.
3. Thaw the cells by submerging the bottom half of the cryovial in 37˚C water bath (do not submerge completely). Swirl the cryovial gently until the cells are almost completely thawed and there is just a small bit of ice left in the vial.
4. Take the cryovial to the biological safety cabinet and wipe the cryovial with 70% EtOH.
5. Using a transfer pipet, add the thawed cells into 8 mL of pre-warmed media in a 50 mL tube.
6. Rinse the cryovials walls with 1 mL of pre-warmed medium and transfer into the tube prepared in previous step.
7. Centrifuge cells at RT for 12 min at 310Xg and discard supernatant.
8. Count the cells based on SOP CBIG Repository: CBIG-03-001 *Different method to count cells*
9. Calculate the total of living and dead cells – if the cell yield is sufficient for your purposes, proceed to next step. If not, consider thawing a second aliquot and pooling them together.
10. Loosen the cap of tube containing the cell suspension; leave it in a 37˚C incubator (5% CO_2) for at least 30 min, but not exceeding two (2) hours. This step may help with increasing cell yield.
11. Count the cells for a second time as per SOP, C-BIG Repository: CBIG-03-001 *Different methods to count cells*, since there might be a reduction in the number of viable cells. Consider this cell count as the final and correct cell density to be used

in future experiments post thawing. While counting, also inspect the cells morphology using inverted microscope.

12. Centrifuge cells at RT for 12 min at 310Xg and discard supernatant.
13. Re-suspend cells to the desired cell density and transfer them to the container of your choice based on your purposes.

7.2 Summary Table

Steps
1.Pre-warmed appropriate medium
2.Thaw the cryovial in a water-bath at 37°C
3.Transfer into a 50 ml tube
4. Centrifuge at RT for 12 min at 310Xg and discard supernatant
5.Count cells
6.Put cell suspension in incubator for at least 30 min and maximum 2 hours
7.Count cells
8.Centrifuge at RT for 12 min at 310Xg and discard supernatant
9.Re-suspend cells and transfer to a container

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

NA

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