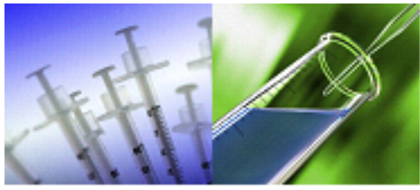

CRYOPRESERVED CELL THAWING

1 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline procedures for thawing cryopreserved cells prepared by the nPOD Organ Procurement and Pathology Core.

2 SCOPE

This SOP will be applied to thawing cryopreserved cells using sterile techniques for optimal cell recovery and functional studies.

3 RESPONSIBILITIES

3.1 Managers and supervisors are responsible for making sure that technicians are properly trained.
3.2 Laboratory personnel are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs and following clinical laboratory and biobanking best practices.

4 EQUIPMENT and MATERIALS

The materials, equipment and forms listed in the following list are recommendations only and alternative products as suitable may be substituted.

Biosafety cabinet
Tabletop centrifuge
Liquid nitrogen cryotank
Hemacytometer with cover glass
Microscope
37^{0}C water bath
Pipette aid
Sterile 15ml conical centrifuge tubes, plastic serological pipettes
Cryovial holder
Dry ice container for transporting cryovials
70% Ethanol
DMEM/F12 media- store refrigerated
Benzonase nuclease (Novagen #70644). Can be substituted with DNAse I solution (Stemcell Technologies # 07900)
Thawing media (DMEM/F12 media+ 50U/ml Benzonase)
Trypan Blue (TB) solution 0.4%

5 SAFETY

5.1 Use universal safety precautions when handling human samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).
5.2 When removing cryovials from a liquid nitrogen freezer, additional eye protective safety glasses and insulated gloves should always be worn.

6 PROCEDURE

6.1 Remove cryopreserved cells from cryoshipper and store in liquid nitrogen cryotank until ready to use.
6.2 Sterile procedures are to be used for those solutions and reagents that contact thawed cells. Do not process more than 3 frozen aliquots at one time so as to work quickly and avoid cell damage. Cells should be thawed quickly and diluted slowly to remove DMSO in cells from the freezing media.
6.3 Prepare supplies and place in biosafety cabinet.
  6.3.1 Label sterile 15ml tube with case ID and sample type (SOP 57 Case Processing).
  6.3.2 Placed pre-warmed thawing media after cleaning bottle exterior with 70% ethanol.
6.4 Remove cell aliquot(s) from the cryotank.
  6.4.1 Transfer vials from liquid nitrogen to a 37°C water bath using dry ice container if needed.
  6.4.2 If liquid nitrogen has seeped into the cryovial, loosen the cap slightly to allow the nitrogen to escape during thawing.
6.5 Hold the cryovial in the surface of the water bath with an occasional gentle “flick”
  6.5.1 Check frequently to monitor thawing. Process may take up to 1 min.
6.6 When there is still a small piece of frozen media, remove from the water bath and spray the vial(s) with 70% ethanol. Dry slightly with a paper towel and place in cryovial holder.
6.7 Open cryovial and transfer contents into a sterile polypropylene 15 ml conical tube using pipette.
6.8 Add thawing media drop-wise while gently swirling to reduce osmotic cell shock.
  6.11.1 Add 0.5 mL drop wise, wait one minute
  6.11.2 Add 1.0 mL drop wise, wait one minute
  6.11.3 Add 2.50mL; gently mix entire cell suspension by pipetting
6.12 Centrifuge tube at 200xg for 7 min. Discard supernatant.
6.13 Gently re-suspend cell pellet with 1 ml of thawing media. Add 5 ml media and centrifuge at 200xg for 7 min.
6.14 Discard supernatant and re-suspend in 1ml of DMEM/F12 media.
6.15 Take 10ul of cell aliquot and proceed with cell counting (SOP 59 Isolation of PBMC).
6.16 Enter cell number into the nPOD Cell Calculator (Excel file) or manually calculate.
6.17 Dilute cell suspension to suitable final working concentration and proceed with assay.

7 REFERENCES

1.1 SOP 57 Case Processing
1.2 SOP 59 Isolation of PBMC
1.3 Garcia-Pineras, M. et al. DNAse treatment following of cryopreserved PBMC is a procedure suitable for lymphocyte functional studies, Journal of Immunological Methods, 2006, 313:209-213

8 REVISION HISTORY

Version	Date	Revision

Prepared by	Irina Kusmartseva		
Approved by	Martha Campbell-Thompson		
	Name	Signature	Date