Department of Medicine Division of Hematology-Oncology Stem Cell and Xenograft Core Standard Operating Procedure		
SOP No.: --- Revision No. - Effective Date: --/--/----	**Title:** **Thawing Viably Frozen Cells**	Page 1of 1
Originator: (sign and date)		
Unit Supervisor: (sign and date)		

1.0 PURPOSE

Instructions for thawing viably frozen cells

2.0 MATERIALS

50 mL conical tubes (polypropylene) - *Catalog# 14-959-49A, Supplier – Fisher*
15 mL conical tubes (polypropylene) - *Catalog# 14-959-70C, Supplier - Fisher*
Cell strainer - *catalog# 22-363-546, supplier – Fisher*
PBS – *Catalog# 14190-235, Supplier – Fisher*
Fetal Bovine Serum – FBS

3.0 PROCEDURE

3.1 Prepare 40mL PBS containing 2% FBS (PBS2) in a 50mL conical tube.
3.2 Place PBS2 in fridge overnight to chill to 4C.
3.3 Take cryotube out of the freezer, spray with 70% ethanol.
3.4 Add 1mL cold PBS to top of frozen cells in cryotube.
3.5 Wait a few minutes to allow cells on top to thaw.
3.6 Keep thawing cells by pipetting back and forth PBS2 between conical tube and cryotube.
3.7 Continue to transfer thawed cells to conical tube.
3.8 Once all cells transferred to conical, centrifuge at 300xg (1200 RPM) for 5 minutes.
3.9 Discard supernatant.
3.10 Resuspend pellet in 50mL conical tube to appropriate volume for cell counting.
3.11 Filter cell using cell strainer to remove dead clumped cells if necessary.
3.12 Counted Cells...see Cell Counting Protocol.
3.13 Centrifuge 300xg=1200RPM for 5 minutes.
3.14 Discard supernatant.
3.15 Resuspend cells in PBS2 and use as needed.