

1.0 Purpose: This document describes the process for the thawing of peripheral blood mononuclear cell (PBMC) samples.

2.0 Scope:

These procedures provide practices for the thawing frozen PBMCs for biobanking studies.

3.0 Definitions/Acronyms:

- 3.1. PBMC: Peripheral Blood Mononuclear Cells
- 3.2. FCS: Fetal Calf Serum

4.0 Requirements:

4.1. Equipment:

- 4.1.1. Centrifuge capable of 200-500xg speeds
- 4.1.2. Water Bath
- 4.1.3. Biosafety Cabinet Hood
- 4.1.4. Pipette Aid

4.2. Materials:

- 4.2.1. 15ml and 50ml Sterile, Polypropylene, Conical, Centrifuge Tubes
- 4.2.2. Sterile pipets

4.3. Reagents:

- 4.3.1. Human AB Serum (must use tested and pre-approved lot) or FCS
- 4.3.2. HL-1 medium

4.4. Method:

4.4.1 Thaw quickly and incompletely by warming the cryovials at 37°C and removing them while a solid core is still visible inside the vial (usually this takes 5 minutes for 1 ml frozen sample to thaw @ 37°C incubator, but only 2 min if a 37°C water bath is used to thaw the cells).

4.4.2 Quickly transfer to a centrifuge tube (15 ml conical tube) containing 4 times prewarmed (25°C) complete HL-1 medium supplemented with 5% human AB serum.

4.4.3 Mix the cells by gentle pipetting and centrifuged for 10 minutes at 330 g at room temperature.

4.4.4 Resuspend the pellet first by tapping the tube, then, in the same complete medium containing 5% human AB serum and ready for use.