PBMC Thawing Protocol

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ABSTRACT

This protocol details methods for thawing peripheral blood mononuclear cells (PBMC).

For a protocol detailing Culture and Stimulation, please view the following: PBMCs Culture and Stimulation.

For a protocol detailing Cell Staining for Flow Cytometry Assay, please view the following: Cell Staining for Flow Cytometry Assay.

ATTACHMENTS

PBMC_Thawing_Protocol.pdf

MATERIALS

Reagents:

- **PBMC washing medium**
  - A: RPMI-1640 with 5 to 10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, 1 mM sodium pyruvate, 2 mM L-glutamine
  - B: 50% X-vivo 15 medium (Lonza) + 25U/ benzonase
  - C: 1X CTL-Anti-Aggregate-Wash™ (CTL)

- **PBMC complete culture medium**
  - RPMI-1640 with 10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, 1 mM sodium pyruvate, 2 mM L-glutamine

Materials:

- 50 mL falcon tube (Fisher scientific #14-432-22)
- Trypan blue
- Water bath
- Dry ice
- 70% EtOH
SAFETY WARNINGS

1. Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

1. Warm Washing buffer and medium to 37 °C in a water bath.

2. Remove vials from liquid nitrogen and transport them to the lab on dry ice.

3. Thaw frozen vials, only 1 vial at a time, in a 37 °C water bath. When cells are nearly completely thawed, carry the vials to the hood and swab them with 70% EtOH.

4. Gently remove PBMCs (avoid pipetting up and down, as the cells are very fragile at this stage) and transfer the cells into a 50 mL falcon tube (Fisher scientific #14-432-22) containing 25 mL warmed washing buffer.

5. Use 1 mL washing medium to rinse out the cryovial and gently mix the cells by inverting the 50 mL Falcon tube ~5x.

6. Wash 1:
   - Spin the cells: 400 x g, Room temperature, 00:08:00. Pour off the supernatant.

7. Wash 2:
   - Suspend the cell pellet in 1 mL prewarmed medium (dropped slowly along the side of the tube) and resuspend the cell pellets, add 9 mL complete medium. Spin the cells:
8 If cells were thawed in the presence of benzonase, perform an additional wash with culture medium in the absence of benzonase.

9 Count cells and determine viability by Trypan blue staining.