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Protocol status: Working
 We use this protocol and it's working

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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

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: 400 x g, Room temperature, 00:08:00 .

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If cells were thawed in the presence of benzonase, perform an additional wash with culture medium in the absence of benzonase.

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Count cells and determine viability by Trypan blue staining.