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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

DOI

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PROTOCOL CITATION

Fang Zhang 2021. PBMC Thawing Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bm9ck92w

KEYWORDS

PBMC, thawing, cell culture, RPMI

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MATERIALS TEXT

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:

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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** .

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.

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