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PBMC Thawing Protocol

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

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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

PDF

PBMC_Thawing_Protoco...

75KB

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 .

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.