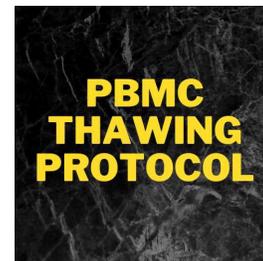


Sep 07, 2021

# 🌐 PBMC Thawing Protocol

DOI

[dx.doi.org/10.17504/protocols.io.bm9ck92w](https://dx.doi.org/10.17504/protocols.io.bm9ck92w)



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DOI: [dx.doi.org/10.17504/protocols.io.bm9ck92w](https://dx.doi.org/10.17504/protocols.io.bm9ck92w)

**Protocol Citation:** Fang Zhang 2021. PBMC Thawing Protocol. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.bm9ck92w>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** October 10, 2020

**Last Modified:** September 07, 2021

**Protocol Integer ID:** 43012

**Keywords:** PBMC, thawing, cell culture, RPMI,

## 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\\_Protoco](#)

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## 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. 