Oct 20, 2017 Version 2

PBMC Isolation V.2

DOI
dx.doi.org/10.17504/protocols.io.kdycs7w

Girija Goyal[1], Jaclyn Long[2]

[1]Wyss Institute, Harvard University; [2]Wyss Institute for Biologically Inspired Engineering at Harvard University

Girija Goyal

DOI: https://dx.doi.org/10.17504/protocols.io.kdycs7w

Protocol Citation: Girija Goyal, Jaclyn Long 2017. PBMC Isolation. **protocols.io https://dx.doi.org/10.17504/protocols.io.kdycs7w**

Protocol status: Working

Created: October 20, 2017

Last Modified: March 25, 2018

Protocol Integer ID: 8344

Keywords: pbmc isolation, peripheral blood mononuclear cells from whole human blood, peripheral blood mononuclear cell, apheresis pack, whole human blood, isolation

Abstract

Commonly used protocol to isolate peripheral blood mononuclear cells from whole human blood or apheresis packs

Guidelines

Objective: Isolate peripheral blood mononuclear cells from fresh whole blood or apheresis packs, also referred to as Leukopaks or collars.In our case, these are platelet-depleted samples of human blood given from a donor. These can vary in volume and cell composition.

Troubleshooting

Safety warnings

Any materials that come into contact with blood should be sterilized with 10% bleach before discarding

Before start

- Make sure to repeatedly label sample with donor number, especially if working with multiple donors
- The protocol here is optimized for 10ml of material from platelet apheresis collars. Variations for other sources have been described.

1 Acquire blood sample from hospital (in our case, from Brigham & Women's Hospital blood donor center)

2 Cut collar and drain blood into 50mL conical tube.

3 Dilute Leukopak with equal volume RPMI or PBS. Mix well. Whole blood does not need to be diluted.

4 Slowly layer solution on top of 10 mL density gradient solution.

5 Centrifuge at 300 g for 20 minutes at room temperature. Set acceleration and deceleration levels to minimal.
22 °C

6 Remove white layer of PBMCs using a 5 mL pipette tip.

7 Add these cells to 10 mL warm media in a 50 mL tube.

8 If using 5 ml or more of the Leukopak, you may have a very high number of cells. To effectively wash them, fill tube to 50 mL.

9 Centrifuge at 300 g for 5 minutes. Return acceleration / deceleration levels to high or 9.

10 Aspirate media and resuspend cells in 20 mL warm media per 10 ml of starting Leukopak. Steps 10-12 can be optimized depending on your yield.

11 For our starting material, dilute cells serially to 1000x. First dilute 100x by adding 10ul cell solutions to 990 ul media in a 1ml eppendorf tube. Then add 10 ul of the 100x dilution to 80 ul media. Add 10 ul trypan blue to this solution.

12 Count cells using a hemocytometer. Count the number of cells in each of the four quadrants. Use the following formula to find the total number of cells.total # of cells = cells counted 4dilution factor 104cellsmltotal volume (ml)

13 Cells can be kept in solution in the refrigerator for up to two hours.