ABSTRACT

List of published work using this protocol


DOI
dx.doi.org/10.17504/protocols.io.biw2kfge
BEFORE STARTING

**MATERIALS TEXT**

- **Fetal bovine serum** (FBS) BioWest Catalog #S181B-500
- **Ficoll Paque PLUS** Ge Healthcare Catalog #17144003-500 ml
- **RPMI** 1640 EuroClone Catalog #ECM 0495L- 500 ml
- **Trypan Blue Solution 0.4%** Thermo Fisher Scientific Catalog #15250061

**Instrumentation required:**

- Laminar flow hood
- Optical Microscope (manual cell count)

**BEFORE STARTING**

If you need to obtain **PBMC for cell culture**, make sure you are using **sterile PBS, culture medium, filtered Lysis Buffer** and **sterile plastic disposables** as well. Moreover, **work under laminar flow hood when you are processing samples**. Otherwise, use non-sterile solutions and plastic disposables, and process samples in cell isolation laboratory.

**ALL REAGENTS USED IN THIS PROTOCOL MUST BE AT ROOM TEMPERATURE!**

1. Put the needed amount of blood sample from buffy coat into a 50 ml conical tube.

2. Add an equal volume of **PBS 1X** and mix well.

3. Place 3 mL of **FICOLL** in a 15 mL conical tube.

4. **CAREFULLY** layer 12 mL of diluted blood on the FICOLL with a glass Pasteur Pipette to a final volume of 15 ml as shown in the figure below.
5 Centrifuge samples 400 x g, 00:40:00 without break.

Allegra AVANTI 30
Centrifuge
Beckman Coulter Beckman Italy

6 After centrifugation, take out the tubes carefully to not disturb the mononuclear cell layer that appears as a white, cloudy band between the plasma and FICOLL as shown in the figure below.
7 Carefully with a glass Pasteur pipette transfer mononuclear lymphocyte cell layer to another 15 ml conical tube.

8 Wash the isolated PBMC with PBS/FBS 2% to a final volume of 10 mL and centrifuge at 300 x g, 00:10:00 at RT.

9 Remove supernatants, resuspend pellet in 1 mL of Lysis Buffer and add another 9 mL of Lysis Buffer. Immediately centrifuge the tubes at 100 x g, 00:10:00 at RT.

10 Remove supernatant and resuspend pellet in 10 mL PBS/FBS 2% and centrifuge at 300 x g, 00:10:00 at RT.
Remove supernatant and resuspend the obtained pellet in 10 mL of RPMI/FBS 10% for cell counting.

For manual cell count use Türk solution for checking purity.

Mix 10 µl of cell suspension with an equal amount of Türk solution (dilution factor = 2), allow mixture 3 min at room temperature. Take 10 µl of the mixture and place it inside a Bürker chamber and view under an optical microscope using 40X magnification.

Count the cells in each square found in the four corners and in the central square (see figure 1 below), including those that lie on the bottom and left-hand perimeters, but not those that lie on the top and right hand perimeters (see figure 2 below).

Total number of cells per ml = mean number of cells x dilution factor x 104 (hemacytometer volume).
The gridded area of the chamber consists of nine 1 mm² squares. These squares are subdivided in three directions; 0.0625 mm², 0.05 mm² and 0.04 mm². The central square here in Figure 1 is further subdivided into 0.0025 mm² = 1/25 mm² squares. Count cells in 5 squares as shown.

Concerning those cells that lay on the perimeter of the square, count following this scheme.

SOLUTION- 08 - Türk solution
by Farmacologia Medica
For automatic cell count with Cellometer machine use Trypan Blue.

The machine will calculate the n° of cells/ml and the % of viability.

Take 10 µl of cell suspention and add an equal amount of Trypan Blue. Use all the volume to place it in a counting chamber. Place the chamber inside Cellometer and count.

If needed, check the purity of PBMC suspension by using morphological parameter of the flow cytometer. For this test 0.5x10^6 PBMC in 500 µl of PBS are enough.

Expected results

VIABILITY - The expected viability by Trypan Blue should be ≥ 90 %.

PURITY - The PBMC suspension obtained should contain at least 80% of lymphocytes, 10-15% of monocytes and few contaminant PMN cells (≤ 5%) as confirmed by flow cytometry.

YIELD - The expected amount of PBMCs should be ± 100x10^6 starting from 25 ml of buffy coat.
Citation: Marco Cosentino, Elisa Storelli, Alessandra Luini, Massimiliano LM Legnaro, Emanuela Rasini, Marco Ferrari, Franca Marino (07/23/2020). PBMC-01a - Isolation of Human PBMC from Buffy Coat. https://dx.doi.org/10.17504/protocols.io.biw2kfqe

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.