Version 2

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LymphocyteCollection V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bnsmmec6

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SUBMIT TO PLOS ONE

ABSTRACT

Purpose: To isolate in separate wells of B lymphocytes and wells of T lymphocytes from fresh whole blood.

Generally, lyse red blood cells and decant to obtain white blood cells. Then, stain with fluorescent antibodies to guide the FACS sort by cell type.

DOI

dx.doi.org/10.17504/protocols.io.bnsmmec6

PROTOCOL CITATION

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WHAT'S NEW

Minor corrections.

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MATERIALS TEXT

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RBC Lysis

Buffer **BioLegend Catalog #420301** In 3 steps

APC anti-human CD19 Clone

SJ25C1 **BioLegend Catalog #392503**

FITC anti-human CD3 Clone

HIT3a **BioLegend Catalog #300305**

BSL2 cabinent
Centrifuge
Serological Pipette
10 ml or 25 ml tips
1 mL, 100ul, and 10ul pipettes
5ml FACS tubes

384 well plate(s); or other container to sort into
Tinfoil - about 8 inches; enough to block light from stained samples

SAFETY WARNINGS

Human blood is BSL2 and should be handled accordingly. This includes working in a biosafety cabinent.

BEFORE STARTING

Schedule FACS machine

Setup

1 Clean biosafety cabinent by UV for 15 minutes, then wipe with Ethanol.

> Human blood requires BioSafety Level 2. We used this protocol with human blood from healthy donors collected from the Student Health Center. Alternatives such as mice blood will have different safety requirements.

Gather pipettes, tubes, racks, and waste container into the cabinent. One person can set up while another is bringing the blood over on ice if two people are available for prep, otherwise leave blood on ice during initial setup.

Old blood (such as from a previous draw) was used for PI+ control, however cells may be otherwise lysed for it.

2 For each control and sample, you will need about **2.4 mL**

PBS **Fisher Scientific Catalog #BP24384**

and **4 mL**

RBC Lysis Buffer **BioLegend Catalog #420301**

Round up when aliquoting from the stock.

Let both stand at room temperature.

We have 10X RBC Lysis Buffer. Prepare the buffer by diluting to 1X with DI Water. For our typical prep of 3 reps x 2 subjects + 4 controls, or 10 tubes, you'll need 25-30 mL PBS and 40-50 mL 1X RBC Lysis Buffer.

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3 Prepare 5ml FACS tubes filled with **4 mL (~4ml)** of

RBC Lysis Buffer **BioLegend Catalog #420301**

at room temp.
1 FACS tube for each replicate - generally 3 per sample
4 more FACS tubes for Controls: Cells Only, FITC only, APC only, PI only (Additional single color controls as necessary.)

4 Vortex the blood if necessary.

Human blood is handled at BioSafety Level 2.

Add blood samples to the corresponding tube:

200 µl of blood for each sample

100 µl of blood for each control

For PI control, use old blood if available; otherwise you will need to lyse or damage the cells

10m

5 Wait **00:03:00** for RBC lysis and then centrifuge **5 rpm, 00:05:00** .

6 Decant into waste bleach solution, blot on paper towel without resuspending.

If red blood cells are still visible in the pellet, add an additional **4 mL (~4ml)** of

RBC Lysis Buffer **BioLegend Catalog #420301** and repeat incubation and centrifugation. Otherwise, the pellet should be white or clear and will be thin.

Lymphocyte staining

7 Prepare staining mix in DARK: (cabinet lights off is sufficient)
Master Mix - Per sample:

100 µl of PBS

PBS **Fisher Scientific Catalog #BP24384**

Add **5 µl** CD3 FITC

FITC anti-human CD3 Clone HIT3a **BioLegend Catalog #300305**

5 µl CD19 APC

APC anti-human CD19 Clone SJ25C1 **BioLegend Catalog #392503**

and **10 µl** PI

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For single color controls, it may be easiest to add **100 µl** PBS followed by the stain directly to the FACS tube, rather than mixing the stain in a separate container first.

8 Add **100 µl** PBS/stain mix to each sample.

9 Incubate cells for **00:15:00** to 20 minutes in the dark, such as covered with tin foil.

10 Add ~ **2 mL** of PBS to each tube,
centrifuge **250 x g, 00:05:00**

11 Decant by dump-tap.
Resuspend in **300 µl** PBS.

FACS prep

12 Filter using 40ul filter prior to any FACS sort. – The cap filters need to be centrifuged, just a minute or two and softly will work. **100 x g, 00:01:00**

Cleanup

13 Wipe the cabinent with ethanol, then turn UV on for **00:15:00**
It may be helpful to turn it on, take the cells for the sort, then come back or have someone else turn it off.

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