VERSION 2

MAR 05, 2021

OPEN ACCESS

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

Protocol Citation: Michaela A McCown, Josie A Tueller, Scott Weber, Samuel H Payne 2021. LymphocyteCollection. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bnsmmec6> Version created by Michaela A McCown

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it works for us.

Created: Oct 22, 2020

Last Modified: Mar 05, 2021

PROTOCOL integer ID:
 43565

LymphocyteCollection V.2

Michaela A McCown¹, Scott Josie A Tueller¹, Weber¹, Samuel H Payne¹

¹Brigham Young University

Michaela A McCown
 Brigham Young University

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.

MATERIALS

MATERIALS

RBC Lysis Buffer BioLegend Catalog #420301

APC anti-human CD19 Clone SJ25C1 BioLegend Catalog #392503

FITC anti-human CD3 Clone HIT3a BioLegend Catalog #300305

BSL2 cabinet

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 cabinet.

BEFORE START INSTRUCTIONS

Schedule FACS machine

Setup

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

Safety information

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 cabinet. 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.

- 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.

Safety information

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

- 5 Wait 00:03:00 for RBC lysis and then centrifuge 5 rpm, 00:05:00 10m

- 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

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 40µl 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 cabinet 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.