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Whole Blood Processing (SepMate)

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1 Works for me dx.doi.org/10.17504/protocols.io.ba87ihzn

Human Cell Atlas Method Development Community

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ABSTRACT

Purpose: To purify PBMCs from 20 or 50mL whole blood (per donor). SepMate tubes will be used with Lymphoprep to ease processing time and effort.

ATTACHMENTS

PBMC isolation from 50 mL whole blood-SepMate Yelab v2 ICC.docx

PBMC isolation from 20 mL whole blood-SepMate Yelab v2 MIB.docx

MATERIALS

NAME	CATALOG #	VENDOR
50ml Conical Tubes, Green Cap, 25/Bag	CT788-G.SIZE.1PK	Bio Basic Inc.
DMSO	D1435	Sigma Aldrich
SepMate™-50 (IVD) 100 Tubes	85450	Stemcell Technologies
Lymphoprep™ 250 mL	7801	Stemcell Technologies
FBS		Invitrogen - Thermo Fisher
Protein LoBind 1.5mL microcentrifuge tubes	0030108116	Eppendorf
EasySep™ Buffer	20144	Stemcell Technologies
Falcon™ 15mL Conical Centrifuge Tubes	14-959-53A	Fisher Scientific
Cryovial 2.0 ml round base internal thread screw cap writing area sterile	121277	greiner bio-one
DPBS, no calcium, no magnesium	14190136	Thermo Fisher
Mr. Frosty Freezing Container, 2mL tubes, Nalgene Mr. Frosty Freezing Container for 1-2mL cryogenic tubes, PC, clear w/ blue lid, 1/Cs.	5100-0001	Thermo Fisher

MATERIALS TEXT

-SepMate-50 (STEMCELL)
-Lymphoprep (STEMCELL)
-EasySep Buffer (STEMCELL)
-DPBS (Ca/Mg free; Fisher)
-15mL conical tubes
-50mL conical tubes
-1.5mL low bind tubes
-ACK lysis buffer
-Freezing media A (100% FBS)
-Freezing media B (20% DMSO in FBS)
-2.0mL Cryovials
-Freezing container

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

BEFORE STARTING

Lymphoprep should be at **Room temperature** (~ **20 °C**) before use. It is important to keep it at **Room temperature** for the density to remain 1.077g/ml.

Plasma Separation

1 Purify 20mL or 50mL whole blood using the following protocol:

step case

50mL whole blood

To purify PBMCs from 50mL whole blood (per donor).
For each donor, 50mL blood will be collected in 5 BD heparin tubes (green top).

2

Centrifuge blood samples at **1200 x g, Room temperature 00:10:00** swinging-out rotor and brake off within **06:00:00** of collection.

3

Carefully transfer **3 ml plasma** from each tube in a 15mL tube avoiding the buffy coat, use remaining blood for PBMC isolation.

Note: To minimize PBMC loss during plasma separation, leave the clear layer level at least 1cm above the RBC.

4

Centrifuge plasma at **16000 x g, 4°C 00:10:00** in fixed-angle rotor (if a high-speed centrifuge for 15mL tubes is not available, aliquot plasma in multiple 1.5mL tubes and centrifuge at **16000 x g**). Transfer plasma to new tubes avoiding the pellet.

5

Aliquot **1 ml plasma** in multiple 1.5mL low bind tubes, freeze at **-20 °C** on the day of collection and move plasma samples to **-80 °C** after **24:00:00** .

PBMC isolation

6 Prepare SepMate tubes with Lymphoprep

6.1

Prepare 3 SepMate tubes per donor and add **15 ml Lymphoprep** below the insert by pipetting into the center hole.

Take care to minimize any air bubbles below the plastic divider.

Video from SepMate manufacturers (~2min)

7

Dilute whole blood 1:2 with DPBS (Ca/Mg free).

For 105mL total: **35 ml residual blood** and **70 ml DPBS** .

8 

Slowly pipette **35 ml diluted blood** down the side of the tube.

Some mixing may occur between the medium and blood, but take care not to mix under the divider.

Unlike conventional gradient separation, do not tilt the tube when adding diluted blood.

9 

Spin at **1200 x g, 20°C 00:15:00** with brakes ON.

Prepare three destination 50mL tubes (~30mL per Sepmate tube will be yielded.)

10 When the spin finishes, pour off the top layer from the SepMate tubes into your prepared destination tubes.

Do not invert the tubes for more than 2 seconds.

11 

Spin at **450 x g, Room temperature 00:10:00** with brake ON.

First wash at higher RCF because gradient medium is mixed with the cells.

12 

Pour off supernatant and combine pellets in three tubes to one, add EasySep buffer to 50mL.

13 

Spin at **350 x g, Room temperature 00:10:00** with brakes ON.

If yield is critical, ensure that the supernatant is clear; opaque supernatant indicates incomplete centrifugation of cells. If necessary, perform the spin at **450 x g** .

14 

Pour off supernatant, wash in **50 ml EasySep buffer** for a second wash.

15 

Spin **350 x g, Room temperature 00:10:00** with brakes on.

16 

Pour off supernatant and resuspend in **20 ml EasySep buffer** .

17 Perform cell count.

18 

Spin at **350 x g 00:10:00** and resuspend in appropriate volume of freezing media A to get 10e7 cells/mL.

19 

Prepare cryovials with **500 µl freezing media B** .

20 

Add **500 µl cell suspension** from previous step to cryovials and gently mix by pipetting.

21 Place in freezing container for 1 day at **-80 °C** and move to LN2 tank for long term storage on the following day.

Plasma Separation

step case

20mL whole blood

To purify PBMCs from 20mL whole blood.
For each donor, 20mL blood will be collected in 2 BD heparin tubes (green top).

2 

Centrifuge blood samples at **1200 x g, Room temperature 00:10:00** swinging-out rotor and brake off within **06:00:00** of collection.

3 

Carefully transfer **3 ml plasma** from each tube in a 15mL tube avoiding the buffy coat, use remaining blood for PBMC isolation.

Note: To minimize PBMC loss during plasma separation, leave the clear layer level at least 1cm above the RBC.