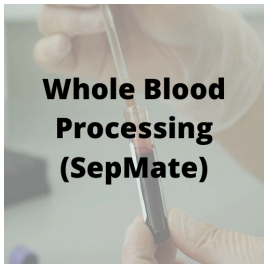
Feb 04, 2020 Version 1

Whole Blood Processing (SepMate) V.1



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Human Cell Atlas Metho...

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Protocol status: Working
We use this protocol and it's working

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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

DOCX
PBMC isolation from ...
17KB

DOCX
PBMC isolation from ...
17KB

Materials

MATERIALS

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

-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

Troubleshooting

Safety warnings

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

Before start

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.

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

STEP CASE

50mL whole blood 21 steps

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

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.

Note

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

Video from SepMate manufacturers (~2min)
https://www.youtube.com/embed/K9IlBxjgLt4

7

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

Note

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

8

Slowly pipette 35 mL diluted blood down the side of the tube.

Note

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.

Note

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.

> Note
>
> Do not invert the tubes for more than 2 seconds.

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

> Note
>
> 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.

> Note
>
> 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.