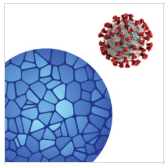

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Purification of PBMCs

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

Human Cell Atlas Method Development Community | Coronavirus Method Development Community

Megan Freund

ABSTRACT

This protocol details the steps and methods of PBMC purification

ATTACHMENTS

PBMCs_isolation_protocol__VENTO_BALLESTAR (1).docx

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ATTACHMENTS

PBMCs_isolation_protocol__VENTO_BALLESTAR (1).docx

MATERIALS TEXT

- Ficoll
- Blood tubes
- 70% ethanol
- 50 mL conical tube(s)
- PBS
- serological pipette
- centrifuge

- pipet tips
- FBS + 10% DMSO

SAFETY WARNINGS

For hazard and safety information, please see the Safety Data Sheet (SDS).

ABSTRACT

This protocol details the steps and methods of PBMC purification

Ficoll Density Gradient Centrifugation

1 Bring Ficoll to **Room temperature** .

2 Clean the blood tubes with 70% ethanol and transfer the blood to a 50mL tube in a BSL2 hood.

3 Dilute blood 1:1 in PBS and mix carefully.

4 Place **15 mL Ficoll** in each of the 50mL conical tubes.

5 

Cover the Ficoll with the blood/PBS mixture using a serological pippette at the lowest speed.

> To avoid compromising the purity of the PBMC, Ficoll and blood should not be mixed in any way. It is therefore best to hold the tube at 45º angle and the blood mixture should exit the pipette and run along the wall of the tube very slowly.

6 

Centrifuge the samples at **800 x g, 20°C, 00:30:00** , selecting acceleration/deceleration rates of 9/1.

> Normally the rotor brakes have to be fully deactivated to effectively prevent phase mixing. It is also very important to strictly adhere to the specified temperatures because temperature differences will change the density rates of the fluids and may have a negative impact on the separation results.

7 After completion of the centrifugation, remove the samples from the centrifuge to avoid phase mixing.

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Lymphocyte Purification

8

Carefully aspirate 2/3 of the top layer (containing the plasma and platelets) using a sterile serological pipette until the interface (containing the mononuclear cells) is within reach.

9

Using a pipette, aspirate the entire lymphocyte layer while keeping the volume minimal and transfer to a clean tube.

Try to transfer as little Ficoll and supernatant as possible during this step.

10

Add at least 3 volumes of PBS to the lymphocyte layer and mix very carefully by pipetting up and down.

11

Centrifuge at **100 x g, 20°C, 00:10:00** and remove the supernatant.

12

Add at least 3 volumes of PBS to the lymphocyte layer and mix very carefully by pipetting up and down.

13

Centrifuge at **100 x g, 20°C, 00:10:00** and remove the supernatant.

14 Resuspend the cells in FBS + 10% DMSO and cryopreserve in liquid nitrogen.

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