PBMCs isolation from CPT™ tube

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1 Works for me This protocol is published without a DOI.

Human Cell Atlas Method Development Community

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ABSTRACT

This protocol details the procedure for collection and isolation of blood samples using CPT tubes.

ATTACHMENTS

PBMCisolation_from_CPTtubes_AIDA.docx

PROTOCOL CITATION

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GUIDELINES

-Composition of Blood preservation solution and condition:

1ml CryosStor(or)DMSO+FBS [DMSO SIGMAD2650HybriMax FBS: Sigma]
AIDA team agrees on commercial Cryostor.

- In our lab, we use Mr. Frosty to drop down the temperature (-1dC/min) once to the -80dC, then put it into liquid nitrogen. (https://www.thermofisher.com/order/catalog/product/5100-0001)

-Store BD Vacutainer® CPT™ Tubes upright at room temperature (18-25° C). Protect tubes from direct light. Shelf life at 18-25ºC is one year from the date of manufacture.

MATERIALS TEXT

Materials:
1) BD vacutainer Cell Preparation Tubes with sodium heparin (Cat. no.362753)
2) FBS Sigma Cat. # F4135
3) ACK-Lysing-Buffer[Thermo,A10492]
4) Phosphate-Buffered Saline (PBS, GIBCO Cat.#10010049)
5) RPMI Medium 1640(GIBCO Cat.11875-093)

Composition of Solutions:
1. Wash buffer (1% FBS, 1 mM EDTA), Store at 4°C
- PBS, pH 7.4 [Gibco, #10010049] 500 mL
- FBS [Sigma] 5 mL
- UltraPure 0.5 M EDTA, pH8.0 [Invitrogen, #15575020] 1 mL

2. ACK Buffer, Store at 4°C
1) Autoclaved MilliQ water 400 mL
2) NH4Cl 4.15 g [154.95328 mM]
3) KHCO 30.5 g [9.99001 mM]
4) UltraPure 0.5 M EDTA, pH8.0 [Invitrogen, #15575020]100 µL [0.09946237 mM]
Mix 1)- 4), adjust pH to 7.2 with 1N HCl and dilute to 500 mL with MilliQ water

SAFETY WARNINGS

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

Excessive centrifuge speed (over 2000 RCF) may cause tube breakage and exposure to blood and possible injury.

BEFORE STARTING

1) The BD Vacutainer® CPT™ Tube should be at room temperature (18-25ºC) and properly labeled for patient identification.
2) After blood collection, store tube upright at room temperature until centrifugation. Blood samples should ideally be centrifuged within two hours of blood collection for best results

Centrifugation and Collection of Blood Sample

1 

Centrifuge tube/blood sample at **Room temperature** in a horizontal rotor (swing-out head) for a minimum of **00:10:00** at **1500 rpm** to **1800 rpm** .

Speed change of accel/decel: Soft

Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times.

2 After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer.

3 Aspirate approximately half of the plasma without disturbing the cell layer.

4 Collect cell layer and transfer to a 50 mL size conical centrifuge tube with cap.

Collection of cells immediately following centrifugation will yield best results.

An alternative procedure for recovering the separated mononuclear cells is to re-suspend the cells into the plasma by inverting the unopened BD vacutainer® CPT™ Tube gently 5 to 10 times. This is the preferred method for storing or transporting the separated sample for up to 24 hours after centrifugation.

5 To collect the cells, open the BD Vacutainer® CPT™ Tube and pipette (use Pasteur Pipette) the entire contents of the tube above the gel into a separate tube.

Cell Washing

6 **First Wash:** Add wash buffer to bring volume to **50 mL** .

7 Cap tube and mix cells by inverting tube 5 times.

Avoid making bubbles if pipetting.

8 

Centrifuge at **300 rpm 00:15:00** .

9 Aspirate as much supernatant as possible without disturbing cell pellet.

10 Re-suspend cell pellet by gently vortexing or tapping tube with index finger.

11 **Second Wash:** Add wash buffer to bring volume to **20 mL** .

12 Cap tube and mix cells by inverting tube 5 times.

13 Re-suspend cell pellet in RPMI 1640 or wash buffer to bring to the concentration of 1×10^{8}cells/mL for counting.

14 Take **10 µl** for cell counting.

14.1 For cell counting, mix the cells with **10 µl Trypan Blue** (pipetting 10 times) and apply **10 µl** of the mixture to a counting slide.

14.2 Count the cells using TCM automatic cell counter within **00:05:00** (the appropriate concentration: 5×10^4~1×10^7cells/mL).

15 

Centrifuge the remaining suspension at **300 rpm 00:10:00** .

16 Aspirate as much supernatant as possible without disturbing cell pellet.

17 Resuspend to freeze down in **1 mL of CryoStor CS10** in cryo tubes.