Section 10.23

TAS General Protocol for Mononuclear Cells Separation

(processing for PBMC)

Aim: Preserving viable cells for research

Purpose: Establishing a technique that is carried out for all research protocols that need PBMC processing and storage.

SPECIAL INSTRUCTIONS: THIS IS A STERILE PROCESSING PROCEDURE, USE STERILE TECHNIQUE

Material

1. PBS (room temperature)
2. PBS (+4*C)
3. CPT tube
4. Centrifuge tube 15 mL
5. Eppendorf tube 1,5 mL

Procedure

Gently invert the CPT tube 5-10 times before starting procedure

1) Centrifuge the CPT tube at. 1500 rcf (2850 rpm on "Beckman" centrifuge) at room temperature for up to 30 min.
2) Obtain the sample number from database and label a 15 mL tube with the number.
3) Label an eppendorf tube (Tracking Number, patient study ID) and put the tube on wet ice.
4) Discard the top layer of serum and transfer the PMBC suspension (usually about 2 mLs) into the 15 mL -entrifuge tube (labeled with corresponded number).
5) Add 10 mL of PBS (room temperature) and mix by inverting.
6) Centrifuge the l5mL tube at -1600 rpm at +4*C "Beckman" for 7) 20 min.
From this point on keep sample on wet ice
8) Aspirate as much supernatant as possible without disturbing cell pellet.
9) Re-suspense the cells pellet in 1 mL of PBS (+4*C).
10) Transfer the cell suspension into the 1,5 mL Eppendorf tube.
11) Centrifuge the Eppendorf tube on centrifuge -2000 rpm at +4*C for 15min.
12) Aspirate as much supernatant as possible without disturbing cell pellet.
13) Snap-freeze the Eppendorf tube with cell pellet in dry ice and put in freezer with under -80* C conditions.