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PBMC Biobanking using Vacutainer® CPT™ Tubes for ChipCytometry Quick Guide

Below are guidelines for separating peripheral blood mononuclear cells (PBMCs) from anticoagulated blood using 8 ml Vacutainer® CPT™ tubes. Cells are loaded onto ZellSafe™ chips and fixed for subsequent biobanking, staining, and ChipCytometry.

A. Blood Collection

1. Prior to the experiment, the BD Vacutainer® CPT™ tubes containing sodium citrate should be stored at room temperature (RT) and properly labeled for patient identification purposes.
2. Draw approximately 6 ml of blood (minimum volume) directly into each 8 ml Vacutainer® CPT™ tube (per 2 chips) using the standard technique for BD Vacutainer Evacuated Blood Collection Tubes.
3. Invert the tubes about 10 times.
4. Store the BD Vacutainer® CPT™ tubes upright at room temp until centrifugation. For optimum results, these samples should be centrifuged within **2 hours** after blood collection.

B. PBMC isolation

1. Mix the BD Vacutainer® CPT™ tubes containing the blood samples immediately before the centrifugation by gently inverting the tubes **8 to 10 times. DO NOT SHAKE.**
2. Place the tubes centered in the swinging bucket, and centrifuge **(20 min; 1600 g; RT)** while keeping the brake ON (Acc. 9, Dec. 9).

Note: You must use a centrifuge with a horizontal rotor (swinging bucket) and an adaptor that can accommodate 16x125 mm tubes.

3. After the centrifugation, the PBMCs are concentrated in a whitish layer just beneath the plasma layer (see Fig. 1).
4. Aspirate approximately half of the plasma using a 1000 µl pipette without disturbing the mononuclear cell band (PBMC layer; see Fig. 1).

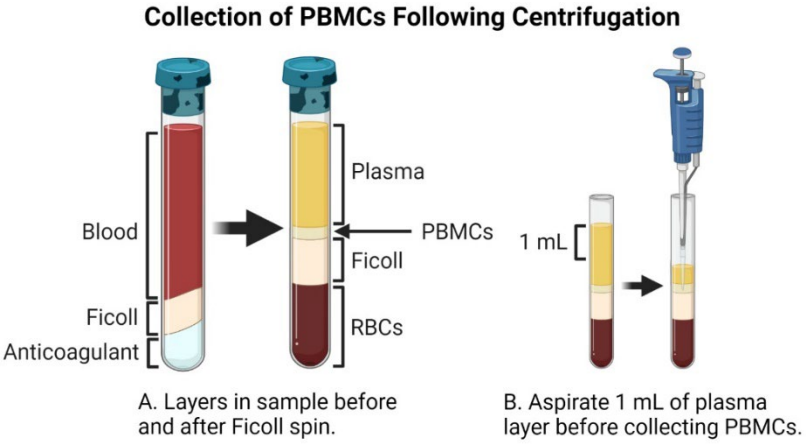

Fig. 1 | Position of PBMCs before and after centrifugation

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B. PBMC isolation continued…

5. Carefully collect **3 times 200 µl** of the PBMC layer with a 200 µl pipette without touching the gel barrier. Transfer this fraction into a BD Falcon round-bottom tube with a cap. For optimum results, collect the PBMCs immediately after the centrifugation.
6. Add **1 ml ZKW wash buffer** and resuspend by pipetting up and down. Close the tube with the cap.
7. Place the tubes centered in the swinging bucket, and centrifuge **(5 min; 100 g; RT)** while keeping the brake ON (Acc. 9, Dec. 9). Do not centrifuge more than 5 min, or at a higher speed, because this causes extensive thrombocyte contamination.
8. Carefully pipette off and discard the supernatant (containing thrombocytes and debris) without disturbing the pellet. Resuspend the pellet in **1 ml ZKW wash buffer** while avoiding the formation of air bubbles.
9. Place the tubes centered in the swinging bucket, and centrifuge again **(5 min; 100 g; RT)** while keeping the brake ON (Acc. 9, Dec. 9).
10. Carefully remove and discard the entire supernatant without disturbing the pellet. Resuspend the pellet in **200 µl ZKW wash buffer.**

C. Preparation and loading of the ZellSafe™ chips

1. Apply the patient identification label on the ZellSafe™ chip at the position indicated in Fig. 2 (optional; not included in the kit). Please do not write on the QR-code label.
2. Place the chip with label side up in the ZKW Washing station. Remove the sealing plug from the inlet of the ZellSafe™ chip (Fig. 3) while leaving the outlet plug sealed. **DO NOT DISCARD SEALING PLUGS AS THEY ARE REUSABLE.**

Fig. 2 | Space for additional label on ZellSafe™ chip (Label not included)

3. Pipette a few drops of **ZKW wash buffer** into the inlet to prevent air from being trapped during pipette adapter insertion.
4. Plug the pipette adapter into the inlet of the ZellSafe™ chip (Fig. 4) and fill the adapter with **ZKW wash buffer** taking care to avoid air bubbles by either directly pipetting with the tip submerged in the liquid in the adapter or hovering the pipette over the adapter and adding wash buffer dropwise.

Fig. 3 | ZellSafe™ chip with sealing plugs blocking the inlet and outlet

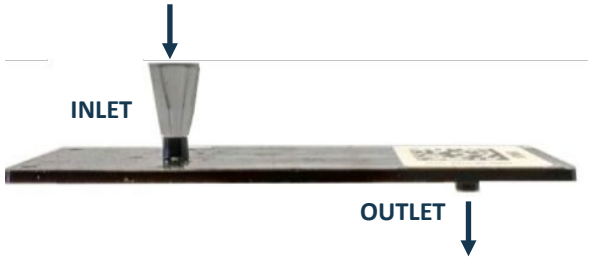

Fig. 4 | ZellSafe™ chip with pipette adapter

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C. Preparation and loading of the ZellSafe™ chips continued…

Note: Air bubbles in the pipette adapter can be removed by carefully aspirating the bubble back into the pipette tip. If an air bubble is visible in the channel, it can be removed by tilting the barcode side of the chip up, inserting the pipette tip all the way into the adapter, and pipetting wash buffer steadily until the bubble exits the channel through the outlet.

DO NOT CLEAR CHANNEL BUBBLES IN THIS MANNER IF UNFIXED CELLS ARE LOADED

5. Remove the sealing plug from the ZellSafe™ chip outlet. Rinse the chip with **3x 200 µl ZKW wash buffer**. Make sure that all air bubbles are removed and that a flow is established before loading the ZellSafe™ chip with cell samples. Once flow is established, pipetting of all solutions (buffers and cell suspension) should be done drop-by-drop.

   Note: Chip should **NEVER** run dry!

6. Pipette 100 µl cell solution into the chip and allow the cells to settle **(5 min; RT).**
7. Rinse the chip with **5x 200 µl ZKW wash buffer** and verify cell density with a standard light microscope (Fig 5).

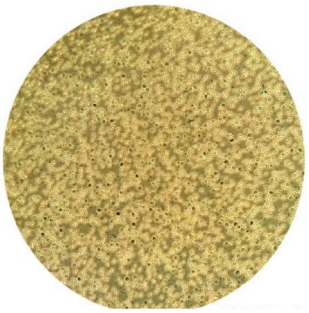

Fig. 5a | Example: Acceptable cell density (*200x*)

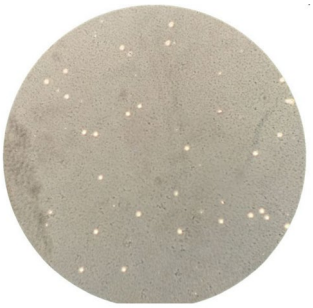

Fig. 5b | Example: Unacceptable cell density (*200x*)

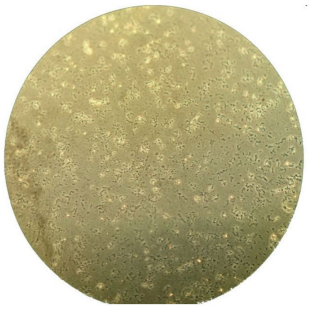

Fig. 5c | Example: Dirt, no cells (*200x*)

8. Rinse the chip with **5x 200 µl ZKW fixation buffer**. Incubate for **45 min at 4°C/ 39°F.**
9. Following incubation, rinse the chip with **5x 200 µl ZKW wash buffer**.
10. For storage, rinse the chip with **5x 200 µl ZKW sterile storage buffer**. Sterile storage buffer should always be used to avoid contamination.

Note: Exchange with fresh, sterile ZKW storage buffer after approximately one year to prevent contamination.

11. Tightly seal the chip with the sealing plugs. Seal the outlet first before sealing the inlet.

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Note: ZellSafe™ chips that are to be shipped should be stored in a ZellSafe™ box. The shipping conditions are 4°C/ 39.2°F with temperature tracking (RFID). **DO NOT FREEZE!**