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Preparation, Prestaining and Biobanking of PBMCs using 8mL Vacutainer® CPT™ Tubes for ChipCytometry Quick Guide

Below are the guidelines for how PBMCs are separated from anticoagulated blood using 8mL Vacutainer® CPT™ tubes and subsequent prestaining, loading and fixation on ZellSafe™ chips for testing.

Note: Clinical bioanalysis requires 6 additional chips for quality control purposes.

When enrolling the first subject in a trial, please draw blood into 3 additional CPT™ tubes, and prepare 6 quality control chips in addition to the study chips. Store unloaded and loaded ZellSafe™ chips in different refrigerators to avoid accidental confusion.

A. Blood Collection

1. Draw 6-7mL of blood directly into each, appropriately labeled, 8mL Vacutainer® CPT™ tubes using the standard technique for BD Vacutainer® Evacuated Blood Collection Tubes.
2. Invert tubes 10 times.
3. Store the BD Vacutainer® CPT™ tubes upright at room temperature until centrifugation. Samples should be centrifuged within **2 hours** following collection.

B. PBMC isolation

1. Immediately before centrifugation, gently invert the BD Vacutainer® CPT™ tubes 8-10 more times. DO NOT SHAKE.
2. Place these tubes on the outer edge of a swinging bucket centrifuge with an adaptor that can accommodate 16x125 mm tubes and centrifuge at 1600 g, RT, for 20 minutes. Keep the brake ON (Acc. 9, Dec. 9).
3. After centrifugation, the PBMCs are concentrated in a whitish layer just beneath the plasma layer (see Fig. 1).

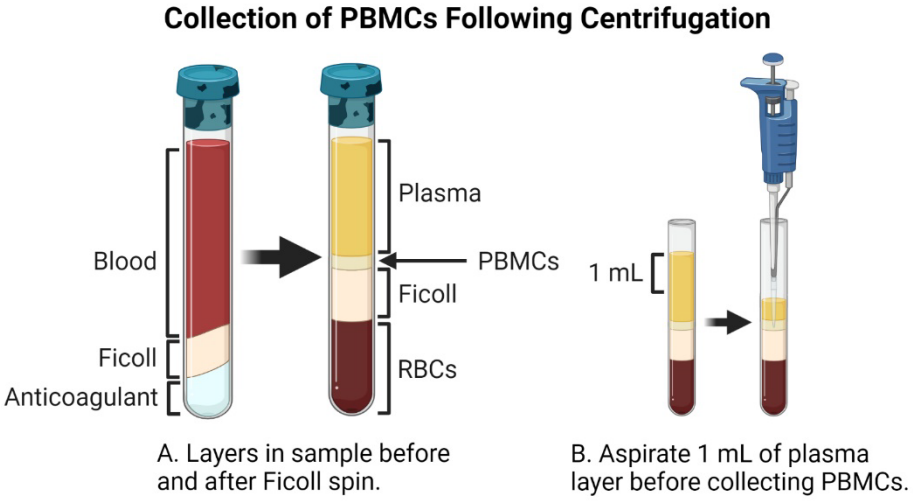

Fig. 1 | Vacutainer pre- and post-centrifugation for PBMC isolation

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B. PBMC Isolation Continued…

3. Aspirate approximately half of the plasma using a 1000µL pipette without disturbing the PBMC layer.
5. Using a 200µL pipette, carefully collect 200µL at a time of the PBMC layer without touching the gel barrier. Transfer this volume into a BD Falcon round-bottom tube with a cap. Continue transferring the PBMC layer until as much as possible has been collected.

Note: If the tip does touch the gel barrier, try to recover as much of the aspirated layer as possible and eject the tip. Start collection again with a fresh tip.

6. Add 1mL ZKW wash buffer to the Falcon tube and resuspend the PBMC layer. Close the tube with the cap.
7. Place the tubes on the outer edge of the swinging bucket centrifuge, and centrifuge 100 g, RT, for 5 minutes while keeping the brake ON (Acc. 9, Dec. 9). **Do not alter the speed or time**, as this will cause thrombocyte contamination.
8. Carefully remove and discard the supernatant (containing thrombocytes and debris) without disturbing the pellet. Resuspend the pellet in 1mL ZKW wash buffer slowly to avoid air bubbles.
9. Place the tubes on the outer edges of the swinging bucket centrifuge, and centrifuge again at 100 g, RT, for 5 minutes while keeping the brake ON (Acc. 9, Dec. 9).
10. Carefully remove and discard the entire supernatant without disturbing the pellet.

C. Prestaining of Cells

1. Prepare the antibody solution. For a 1:30 dilution, add 290µL ZKW storage buffer and 10µL of the antibody stock to a 1.5mL Eppendorf tube. Vortex.
2. Add the whole volume to the cell pellet, resuspend and incubate for 5 minutes at RT.
3. Centrifuge for 5 minutes at 300 g with the brake ON.
4. Carefully remove and discard the entire supernatant without disturbing the pellet. Resuspend the pellet in 300µL ZKW wash buffer.
5. Centrifuge for 5 minutes at 300 g with the brake ON.
6. Carefully remove and discard the entire supernatant without disturbing the pellet. Resuspend pellet in 200µL ZKW wash buffer.

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D. Preparation and Loading of the ZellSafe™ Chips

1. Apply the patient identification label on the ZellSafe™ chip at the position indicated in Fig. 2a (optional; not included in the kit). Please do not write on the QR-code label.

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

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. 2b) while leaving the outlet plug sealed. **DO NOT DISCARD SEALING PLUGS AS THEY ARE REUSABLE.**

Fig. 2b | ZellSafe™ chip with sealing plugs blocking the inlet and outlet

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. 2c) 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.

   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: The chip should **NEVER** run dry!

Fig. 2c | ZellSafe™ chip with pipette adapter

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D. Preparation and Loading of the ZellSafe™ Chips Continued…

Note: The 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 3).

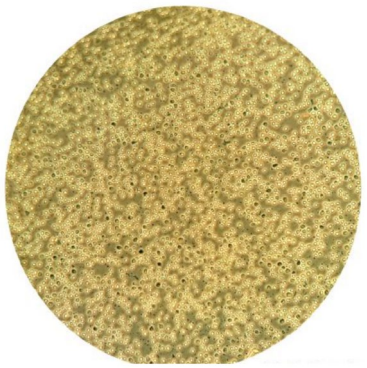

Fig. 3a | Example: Acceptable cell density (*200x*)

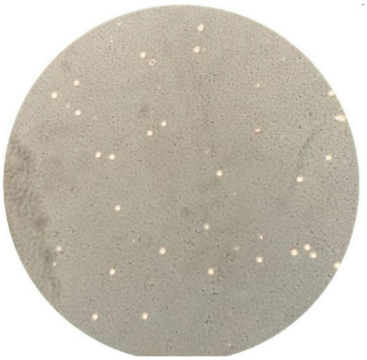

Fig. 3b | Example: Unacceptable cell density (*200x*)

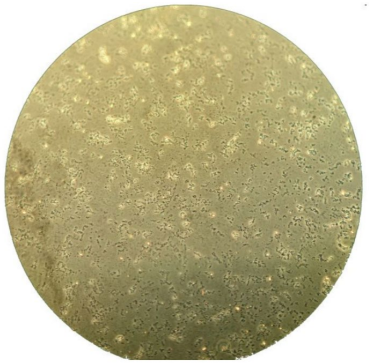

Fig. 3c | Example: Dirt, no cells (*200x*)

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 200µL ZKW wash buffer.
10. For storage, rinse the chip with 5x 200 µL ZKW storage buffer. Sterile storage buffer should always be used to avoid contamination.

Note: Exchange with fresh, sterile ZELLKRAFTWERK 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.

    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!

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