A BRUKER COMPANY
CANOPY BIOSCIENCES

4340 Duncan Ave | Suite 220
Saint Louis, MO | 63110
+1 (314) 833-9764

PBMC Biobanking using Ficoll™ Tubes for ChipCytometry Quick Guide

Below are guidelines for separating peripheral blood mononuclear cells (PBMCs) from anticoagulated blood using Ficoll™ tubes. Cells are loaded onto ZellSafe™ chips and fixed for subsequent biobanking, staining, and ChipCytometry.
Note: 1mL anticoagulated blood is sufficient to load one ZellSafe™ chip.

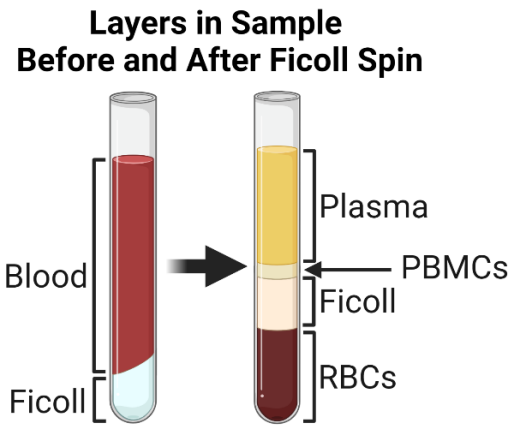

Fig. 1 | Layers in Ficoll™ tubes pre- and post-centrifugation
Figure created using BioRender

A. PBMC isolation

1. Pipette 1.5 ml Ficoll™ to a FACS-tube.
2. Dilute 1 ml anticoagulated blood with **1 ml ZELLKRAFTWERK wash buffer** and CAREFULLY layer the diluted blood on top of the Ficoll™ phase. Do not mix.
3. Place the tubes centered in a swinging bucket and centrifuge **(10 min; 465 g; RT; Acc. 7; Dec. 1)**.
4. After the centrifugation, the PBMCs are concentrated in a whitish layer just beneath the plasma layer (see Fig. 1).

Note: A reddish PBMC layer indicates contamination with red blood cells and reduced sample quality.

5. Carefully collect the PBMC layer with a pipette.
6. Transfer this fraction into a new FACS tube. For optimal results, collect the PBMCs immediately after the centrifugation.
7. Add **1 ml ZELLKRAFTWERK wash buffer** and resuspend by pipetting up and down. Close the tube with the cap.
8. 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.
9. Carefully remove and discard the supernatant (containing thrombocytes and debris) without disturbing the pellet. Resuspend the pellet in **1 ml ZELLKRAFTWERK wash buffer** while avoiding the formation of air bubbles.
10. 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).
11. Carefully remove and discard the entire supernatant without disturbing the pellet. Resuspend the pellet in **100 µl ZELLKRAFTWERK wash buffer.**

V1.0_Updated Jun 2021

ChipCytometry PBMC Quick Guide

B. 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 ZELLKRAFTWERK 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.**

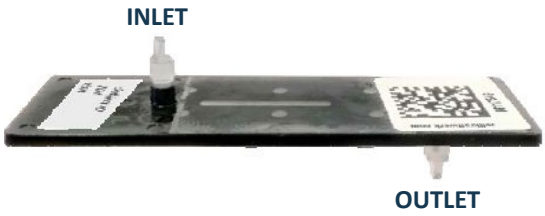

Fig. 2b | ZellSafe™ chip with sealing plugs blocking the inlet and outlet

3. Pipette a few drops of **ZELLKRAFTWERK 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 **ZELLKRAFTWERK 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. 2c | ZellSafe™ chip with pipette adapter

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 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 ZELLKRAFTWERK 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!
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 ZELLKRAFTWERK wash buffer** and verify cell density with a standard light microscope (Fig 3).

A BRUKER COMPANY
CANOPY BIOSCIENCES

ChipCytometry PBMC Quick Guide

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

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

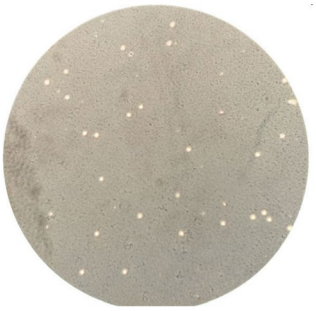

Fig. 3b | Example:
Unacceptable cell density
(*200x*)

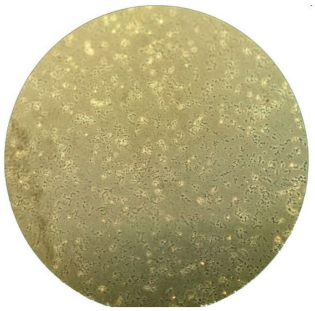

Fig. 3c | Example:
Dirt, no cells
(*200x*)

8. Rinse the chip with **5x 200 µl ZELLKRAFTWERK fixation buffer**. Incubate for **45 min at 4°C/ 39°F.**
9. Following incubation, rinse the chip with **5x 200 µl ZELLKRAFTWERK wash buffer**.
10. For storage, rinse the chip with **5x 200 µl ZELLKRAFTWERK sterile 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!**

A BRUKER COMPANY
CANOPY BIOSCIENCES