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

Leukocyte Preparation by Erythrocyte Lysis for ChipCytometry Quick Guide

Below are guidelines for isolation of leukocytes from EDTA-anticoagulated blood by erythrocyte lysis for subsequent biobanking, staining, and ChipCytometry.

Do not use heparin-anticoagulated blood as it will result in an incomplete lysis of erythrocytes

Fig. 1 | Space for additional label on ZellSafe™ chip (Label not included)

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

A. Erythrocyte Lysis

1. Create a 1X working concentration from 10X RBC lysis buffer and deionized water (e.g. 1800 µl DI water + 200 µl 10X RBC lysis buffer)
2. Add 2000µl of this 1X RBC lysis buffer to 100µl of EDTA-anticoagulated blood in an appropriate tube.
3. Gently vortex and incubate in the dark, at RT, for 15 minutes.
4. Centrifuge this suspension (10 minutes, 350xg, RT) while keeping the brake ON (acc. 9, dec. 9).
5. Carefully remove and discarded the supernatant without touching the pellet.
6. Resuspend this pellet in 500µl ZKW wash buffer.
7. Centrifuge this suspension (10 minutes, 350xg, RT) while keeping the brake ON (acc. 9, dec. 9).
8. Carefully remove the supernatant completely and resuspend the pellet in 40 µl ZKW wash buffer.

B. Preparation and loading of the ZellSafe™ chips

1. Apply the patient identification label on the ZellSafe™ chip at the position indicated in Fig. 1 (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 ZELLKRAFTWERK Washing station. Remove the sealing plug from the inlet of the ZellSafe™ chip (Fig. 2) while leaving the outlet plug sealed. **DO NOT DISCARD SEALING PLUGS AS THEY ARE REUSABLE.**
3. Pipette a few drops of **ZELLKRAFTWERK wash buffer** into the inlet to prevent air from being trapped during pipette adapter insertion.

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ChipCytometry Leukocyte Prep Quick Guide

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

4. Plug the pipette adapter into the inlet of the ZellSafe™ chip (Fig. 3) 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.

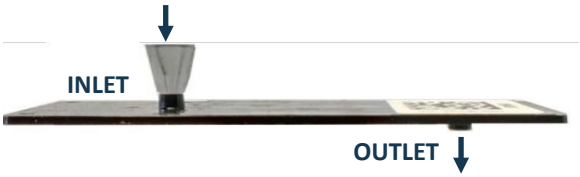

Fig. 3 | ZellSafe™ chip with pipette adapter

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 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 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. **The chip should NEVER run dry!**
6. Pipette 40 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 4).

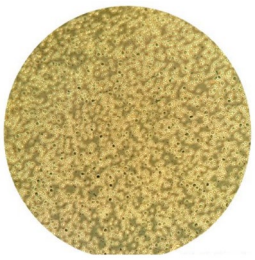

Fig. 4a | Example: Acceptable cell density (*200x*)

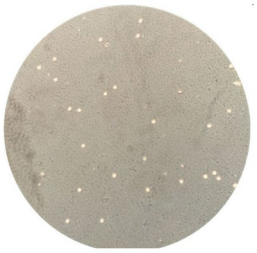

Fig. 4b | Example: Unacceptable cell density (*200x*)

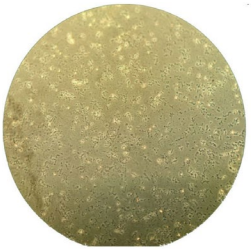

Fig. 4c | Example: Dirt, no cells (*200x*)

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

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

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!**