IFU – WBC Adhesion on SmartBioSurface® Slides (RUO)

Intended Use/Destination of Use (for Research Use Only)

This Instructions for Use (IFU) describes the procedure for processing fresh EDTA whole blood samples (without preservative) by red blood cell lysis to obtain a white blood cell (WBC) suspension suitable for spontaneous adhesion onto SmartBioSurface® slides for downstream research applications [1-3].

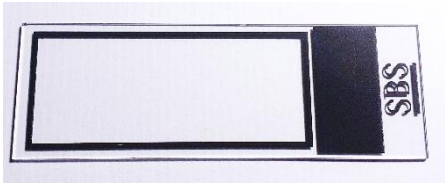
The device does not provide specific results as it is involved in the treatment process aimed at preparing the sample for the next step; therefore, the device is an aid to diagnosis and does not provide any interpretable analytical results.
Slides in intact box and package have a stability of 18 months.

Red Blood Cell Lysis

1. Transfer 8 mL of whole blood into a conical tube. *(If a different blood volume is used, adjust all reagent volumes proportionally while maintaining the same blood-to-buffer ratio.)*
2. Determine the leukocyte count in the whole blood sample using an automated hematology analyzer. *(If an automated hematology analyzer is not available, determine the WBC concentration manually after red blood cell lysis and resuspension in 1× DPBS using a hemocytometer or an equivalent cell counting method.)*
3. Prepare 1× RBC Lysis Buffer by diluting the 10× RBC Lysis Buffer Stock Solution (PluriSelect, REF 60-00051-10) with Milli-Q water according to the manufacturer's instructions.
4. Add 1× RBC Lysis Buffer to the whole blood sample until a final volume of 28 mL is reached. Mix by pipetting up and down five times using a 10 mL serological pipette. Do not invert the tube.
5. Incubate the sample for 5 minutes at room temperature (RT).
6. Mix the suspension again by pipetting up and down five times using a 10 mL serological pipette. Do not invert the tube.
7. Verify that red blood cell lysis is complete. The suspension should change from opaque and turbid to clear. If lysis is incomplete, continue pipetting until the suspension becomes completely clear. Do not centrifuge the sample before completing red blood cell lysis.
8. Centrifuge at 1,400 rpm for 5 minutes.
9. Remove the supernatant as follows:
    a. Aspirate 23 mL using a 10 mL serological pipette, leaving 5 mL in the tube.
    b. Remove an additional 3 mL using a P1000 pipette.
    c. Add 1 mL of fresh 1× RBC Lysis Buffer and resuspend the pellet by pipetting at least three times.
10. Add 12 mL of fresh 1× RBC Lysis Buffer to obtain a final volume of 15 mL.
11. Centrifuge at 1,400 rpm for 5 minutes.
12. Remove the supernatant as follows:
    a. Aspirate 10 mL using a 10 mL serological pipette, leaving 5 mL in the tube.
    b. Remove an additional 4 mL using a P1000 pipette.
    c. Add 1 mL of fresh 1× RBC Lysis Buffer and resuspend the pellet by pipetting at least three times.
    d. Add 13 mL of fresh 1× RBC Lysis Buffer to obtain a final volume of 15 mL.
13. Centrifuge at 1,400 rpm for 5 minutes.
14. Remove the supernatant as follows:
    a. Aspirate 10 mL using a 10 mL serological pipette, leaving 5 mL in the tube.
    b. Remove any remaining supernatant using a P1000 pipette.
    c. Resuspend the pellet thoroughly in 1 mL of 1× DPBS.

Note: *After resuspension in 1× DPBS, proceed to the seeding step without delay. Cells should not remain in 1× DPBS for longer than 30 minutes, as extended exposure may compromise cell viability.*

White Blood Cell Adhesion and Fixation on SmartBioSurface slides



1. Open the SmartBioSurface slide package and record the opening date on the box. Use the slides within 1 month after opening.
2. Remove the slides from the package and identify the active side, characterized by the black Teflon chamber. The sample deposition area consists of a 20 × 50 mm transparent chamber confined by a 30 µm black Teflon frame and coated with a 50 nm nanostructured titanium dioxide (TiO_2) layer designed to promote spontaneous cell adhesion.
3. Do not touch the sample deposition area. Remove the slides from the package only when the WBC suspension is ready to be deposited. Label the slides if required.
4. Based on the total WBC count, resuspend the WBC pellet in 1× DPBS to obtain the appropriate cell concentration for seeding onto SmartBioSurface slides. *(The resuspension volume should be calculated according to the number of SmartBioSurface slides to be prepared. A standard analytical unit for CTC analysis consists of 10 slides.)*
5. Gently invert the tube containing the WBC suspension three times to ensure homogeneous cell distribution.
6. Pipette the WBC suspension into the center of each SmartBioSurface adhesion area.
    a. Minimum deposition volume: 250 µL
    b. Maximum deposition volume: 800 µL
    c. Recommended deposition volume: 800 µL
    d. Maximum cell number: 2.5 × 10^6 WBCs per adhesion area

Example calculation of the 1× DPBS resuspension volume:

$$\frac{\text{WBC concentration (cells/mL)} \times \text{Blood volume (mL)}}{2.5 \times 10^6 \text{ cells/slide}} = \text{Number of SmartBioSurface slides}$$

$$\text{Number of SmartBioSurface slides} \times 0.8 \text{ mL/slide} = \text{Final 1× DPBS resuspension volume (mL)}$$

7. Leave the slides undisturbed for 20 minutes at room temperature (RT) to allow spontaneous cell adhesion.
8. Remove the supernatant by pipetting or by gently tilting each slide.
9. Gently immerse the slides in 4% paraformaldehyde (PFA) in 1× DPBS for 20 minutes at room temperature (RT).
10. Gently transfer the slides to 1× DPBS and wash for 30 seconds.
11. Remove the slides from the 1× DPBS solution and gently dry the back and edges using absorbent paper.
12. Leave the slides to air dry overnight under a chemical fume hood.
13. Once completely dry, proceed with staining or store the slides at –80 °C.

Fig. 1. Image of a SmartBioSurface slide with 800 µL of cell solution pipetted the cell deposition areas.

References

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2. Krol I, Schwab FD, Carbone R, Ritter M, Picocci S, De Marni ML, Stepien G, Franchi GM, Zanardi A, Rissoglio MD, Covelli A, Guidi G, Scarinci D, Castro-Giner F, Mazzarella L, Doglioni C, Borghi F, Milani P, Kurzeder C, Weber WP, Aceto N. Detection of clustered circulating tumour cells in early breast cancer. Br J Cancer. 2021 Jul;125(1):23-27. doi: 10.1038/s41416-021-01327-8. Epub 2021 Mar 24. PMID: 33762721; PMCID: PMC8257701.
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