

Whole blood processing kit

P01 - Sample stabilisation



Preparation phase:

Aliquot cryogenic vials¹ with **Cytodelics Stabiliser equal the planned blood sample volume. Store at 5–8°C until sampling (up to 2 years). Identify dispensed volume on the label.**

Protocol:

1. Bring tubes pre-filled with the Stabiliser **to room temperature** (5 – 10 minutes).
2. Transfer equal **blood** volume to the cryogenic vial (RATIO 1:1).
3. **Mix** the blood sample with the Stabiliser **by flipping** the vial 10-15 times. **Do not** vortex!
4. Incubate at room temperature for 10 minutes.
5. Store the sample until further processing at:
 - a. $\leq -80^{\circ}\text{C}$ (up to 1 year);
 - b. $\leq -20^{\circ}\text{C}$ (up to 2 months), then transfer to the ultra-low freezer ($\leq -80^{\circ}\text{C}$) as soon as possible and keep there until further processing (up to 1 year);
 - c. or place on **dry ice** and transfer to to the ultra-low freezer ($\leq -80^{\circ}\text{C}$) as soon as possible and keep there until further processing (up to 1 year).

¹ Choose cryogenic vial with nominal volume of approx. 3-times of the planned blood sample.

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P01 - Processing after sample thawing

1. **Calculate** the required volume of the **Fixation Buffer** (total volume of blood processed within one batch multiplied by factor 10) and **prepare** it by mixing equal volumes of
 - a. Fix-concentrate
 - b. Fix-Diluent
2. **Dispense** the **Fixation Buffer** (10-times volume of preserved blood; do not count on the Stabiliser volume²) to tubes and let to equilibrate to room temperature (approx. 5 minutes).
3. **Thaw** blood samples preserved in the Stabiliser by quickly heating on water bath or heat block set to 37°C (approx. 1 minute).
4. **Transfer** {blood+Stabiliser} mixture to the tube with the Fixation buffer, set the timer, and **incubate** for **15 minutes** at **room temperature**. Vortex multiple times throughout.
5. **Add** 1-time concentrated **LYSIS buffer** (40-times volume of the blood sample³), **mix** well by inverting tube multiple times, and **incubate** at room temperature for minimum of **15 to 20 minutes** until a complete erythrocytes' lysis.
6. **Centrifuge** at **300g for 5 to 10 minutes**⁴.
7. **Aspirate the supernatant**, according to the size of the sample, down to:
 - approx. mark 5.0-7.5 ml in 50mL tube, and before the next step, proceed as follows:
 - i. transfer to a 15mL tube,
 - ii. rinse twice with 2.0 ml of the **LYSIS buffer** and transfer to the 15mL tube,
 - iii. centrifuge at 300g for 5 minutes,
 - iv. aspirate the remaining volume down to approx. mark 0.5 ml.



² E.g., for 1.0 ml of blood preserved in 1.0 ml of Stabiliser use **10.0 ml of the Fixation Buffer**, i.e., 10-times volume of the blood.

³ E.g., for 1.0 ml of blood and 10.0 ml of Fixation Buffer use **40.0 ml of the LYSIS buffer**.

⁴ Set the centrifugation timing according to the liquid column height in the tube and rotor size. The timing should increase with shorter rotor diameter and/or more liquid (higher liquid column) per tube.

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- approx. mark 0.5 ml in a 15mL tube (for samples with original volume up to 250µl blood processed in the 15mL tube from the beginning),
 - “ring” on the 5mL “FACS” tube, leaving a minimum of 100 µl of supernatant over the pellet.
8. Break the pellet by vortexing and **add** 1-time concentrated **WASH buffer** (40-times the volume of the blood sample⁵).
 9. **Centrifuge at 300g for 5 to 10 minutes**⁶.
 10. **Gently aspirate** the supernatant down to:
 - a. approx. mark 0.25 ml (15mL tube)
 - b. ring” on the 5mL “FACS” tube leaving approx. 100 µl of supernatant over the pellet.

Do not decant!

11. Proceed with:
 - a. sample staining and acquisition,
 - b. **cryogenic preservation** in CRYO#20 buffer at $\leq -80^{\circ}\text{C}$.

For thawing, proceed as follows:

- i. Thaw the sample in a range from 20°C to 37°C (ideally on a heat block),
- ii. Process according to your downstream application⁷.



⁵ E.g., for 1.0 ml of blood, 10.0 ml of Fixation Buffer, and 40ml of LYSIS buffer use **40.0 ml of the WASH buffer**.

⁶ Set the centrifugation timing according to the liquid column height in the tube and rotor size. The timing should increase with shorter rotor diameter and/or more liquid (higher liquid column) per tube.

⁷ You may stain without the washing step and acquire; however, we recommend to test compatibility of your staining panel. Before the staining, centrifugate the sample at 300-400g/ \approx 5 min and remove some of the supernatant to optimize volume of the Ab-cocktail and antibodies consumption.

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P02 - Staining of fixation-sensitive epitopes

1. **Prepare staining cocktail:**
 - a. complete Ab-panel,
 - b. containing antibodies targeting fixation-sensitive epitopes.
2. Mix **fresh blood** sample with the **Ab-cocktail** and incubate for 30-60 minutes at temperatures ranging from 5°C to 37°C according to your preference and conditions recommended by the antibodies' producers.

Meanwhile:

- a. **Calculate** the required volume of the **Fixation Buffer** (total volume of blood processed within one batch multiplied by factor 10) and **prepare** it by mixing equal volumes of
 - i. Fix-concentrate
 - ii. Fix-Diluentand let to equilibrate to room temperature (approx. 5 min).
3. **Dispense** the **Fixation Buffer** (10-times volume of stained blood¹) to tubes with stained blood samples, set the timer, and **incubate** for **15 minutes** at **room temperature**. Vortex multiple times throughout.
 4. **Add** 1-times concentrated **LYSIS buffer** (40-times volume of the blood sample²), **mix** well by inverting tube multiple times, and **incubate** at room temperature for minimum **15 to 20 minutes** until a complete erythrocytes lysis.
 5. **Centrifuge** at **300g for 5 to 10 minutes**³.
 6. **Aspirate supernatant** down to:
 - a. “ring” on the 5mL “FACS” tube, leaving a minimum of 100 µl of supernatant over the pellet,

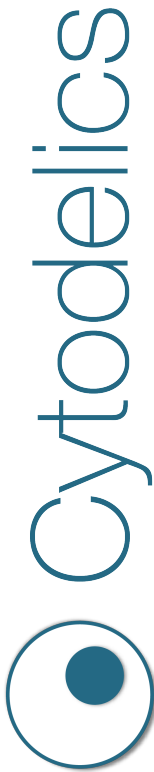
¹ E.g., for 100 µl of blood use **1.0 ml of the Fixation Buffer**.

² E.g., for 100 µl of blood and 1.0 ml of Fixation Buffer use **4.0 ml of the LYSIS buffer**.

³ Set the centrifugation timing according to the liquid column height in the tube and rotor size. The timing should increase with shorter rotor diameter and/or more liquid (higher liquid column) per tube.

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- b. approx. mark 0.5 ml in 15mL tube (for samples with original volume up to 250µl blood processed in the 15mL tube from beginning),
 - c. approx. mark 5.0-7.5 ml in 50mL tube, and before the next step, proceed as follows:
 - i. transfer to a 15mL tube
 - ii. centrifuge at 300g for 5 minutes,
 - iii. aspirate the remaining volume down to approx. mark 0.5 ml.
 7. Break the pellet by vortexing and **add** 1-time concentrated **WASH buffer** (40-times volume of the blood sample⁴).
 8. **Centrifuge at 300g for 5 to 10 minutes**⁵.
 9. **Gently aspirate** the supernatant down to:
 - a. approx. mark 0.25 ml (15mL tube)
 - b. ring” on the 5mL “FACS” tube leaving approx. 100 µl of supernatant over the pellet.
- Do not decant!**
10. Proceed with:
 - a. **acquisition**
 - b. **cryogenic preservation** in CRYO#20 buffer at $\leq -80^{\circ}\text{C}$.
For thawing, proceed as follows:
 - i. Thaw the sample in a range from 20°C to 37°C (ideally on a heat block),
 - ii. Acquire ⁶ samples or continue with staining procedure without washing⁷ step.



⁴ E.g., for 100 µl, 1.0 ml of Fixation Buffer, and 4.0 ml of LYSIS buffer use **4.0 ml of the WASH buffer**.

⁵ Set the centrifugation timing according to the liquid column height in the tube and rotor size. The timing should increase with shorter rotor diameter and/or more liquid (higher liquid column) per tube.

⁶ Keep the volume of the CRYO#20 buffer unchanged or optimise it according to your flow cytometer specifications.

⁷ Centrifugate the sample at 300-400g/≈5 min and remove some of the supernatant to optimize volume for staining with Ab-cocktail

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P03 - Fresh blood fixation

1. **Calculate** the required volume of the **Fixation Buffer** (total volume of blood processed within one batch multiplied by factor 10) and **prepare** it by mixing equal volumes of
 - a. Fix-concentrate
 - b. Fix-Diluent
2. **Dispense** the **Fixation Buffer** (10-times volume of preserved blood) to tubes and let it equilibrate to room temperature (approx. 5 min).
3. **Transfer** blood samples to the tube with the Fixation buffer, set the timer, and **incubate** for **15 minutes** at **room temperature**. Vortex multiple times throughout.
4. **Add** 1-times concentrated **LYSIS buffer** (40-times volume of the blood sample¹), **mix** well by inverting tube multiple times, and **incubate** at room temperature for minimum **15 to 20 minutes** until a complete erythrocytes' lysis.
5. **Centrifuge** at **300g for 5 to 10 minutes**².
6. **Aspirate the supernatant** down to:
 - a. "ring" on the 5mL "FACS" tube leaving minimum 100 µl of supernatant over the pellet,
 - b. approx. mark 0.5 ml in 15mL tube (for samples with original volume up to 250µl blood processed in the 15mL tube from beginning),
 - c. approx. mark 5.0-7.5 ml in 50mL tube, and before the next step, proceed as follows:
 - i. transfer to a 15mL tube
 - ii. centrifuge at 300g for 5 minutes,
 - iii. aspirate the remaining volume down to approx. mark 0.5 ml.
7. Break the pellet by vortexing and **add** 1-time concentrated **WASH buffer** (40 times the volume of the blood sample³).



¹ E.g., for 1.0 ml of blood and 10.0 ml of Fixation Buffer use **40.0 ml of the LYSIS buffer**.

² Set the centrifugation timing according to the liquid column height in the tube and rotor size. The timing should increase with shorter rotor diameter and/or more liquid (higher liquid column) per tube.

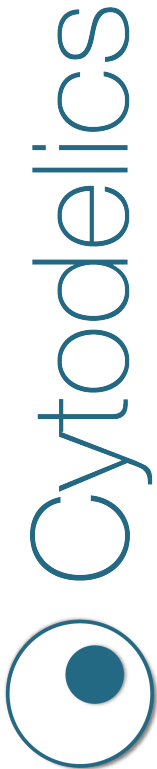
³ E.g., for 1.0 ml of blood, 10.0 ml of Fixation Buffer, and 40ml of LYSIS buffer use **40.0 ml of the WASH buffer**.

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8. **Centrifuge at 300g for 5 to 10 minutes**⁴.
9. **Gently aspirate** the supernatant down to:
 - a. approx. mark 0.25 ml (15mL tube)
 - b. ring” on the 5mL “FACS” tube leaving approx. 100 µl of supernatant over the pellet.

Do not decant!

10. Proceed with:
 - a. **acquisition**
 - b. **cryogenic preservation** in CRYO#20 buffer at $\leq -80^{\circ}\text{C}$.
For thawing, proceed as follows:
 - i. Thaw the sample in a range from 20°C to 37°C (ideally on a heat block),
 - ii. Acquire ⁵ samples or continue with staining procedure without washing⁶ step.



⁴ Set the centrifugation timing according to the liquid column height in the tube and rotor size. The timing should increase with shorter rotor diameter and/or more liquid (higher liquid column) per tube.

⁵ Keep the volume of the CRYO#20 buffer unchanged or optimise it according to your flow cytometer specifications.

⁶ Centrifugate the sample at 300-400g/ \approx 5 min and remove some of the supernatant to optimize volume for staining with Ab-cocktail.