P04 – Fresh blood fixation: quick protocol

Text version of the graphical quick protocol. For fixation of fresh whole blood with the Whole Blood Fix/Lyse Kit. Detailed instructions, tube options and volumes: P03 – Fresh blood fixation.

Protocol

1. **Prepare 1x Fixation buffer:** mix 1 part of **2x Fix-Concentrate** with 1 part of **Fix-Diluent** (total volume = 10-times the blood volume), dispense into test tubes and let equilibrate at room temperature for **5 minutes**.
2. Transfer the **blood** to the tube with the Fixation buffer.
3. **Incubate** at room temperature (approx. 20˚C) for **15 minutes**. Vortex multiple times throughout.
4. Add **LYSIS buffer** (40-times the blood volume) and mix by vortexing or inverting.
5. **Incubate** at room temperature for **15 – 20 minutes**, until the solution is crystal clear red (see the guide below). **Do not proceed without complete lysis of red blood cells!**
6. **Centrifuge** at **300g for 5 – 10 minutes**.
7. Aspirate the supernatant, leaving some volume at the bottom (min. 100 µl). **Do not decant!**
8. Break the pellet by vortexing and add **WASH buffer** (40-times the blood volume).
9. **Centrifuge** at **300g for 5 – 10 minutes**.
10. Aspirate the supernatant, leaving some volume at the bottom. **Do not decant!**
11. If the pellet still contains red blood cells (pinkish-red), repeat steps 8 – 10.
12. Proceed with either:
    a. staining and acquisition of the sample, or
    b. cryogenic preservation in **CRYO#20** (0.5 – 1-times the blood volume) at **≤ -80˚C** (see P09).

Scaling ratios			
Blood	1	10	Fixation buffer
Blood	1	40	LYSIS buffer
Blood	1	40	WASH buffer
Blood	1	0.5 - 1	CRYO#20

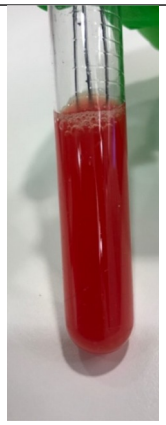
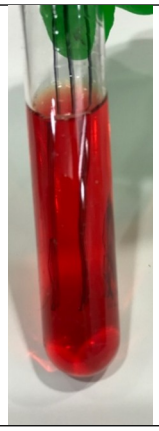
Examples of recommended processing conditions

Blood sample volume	Fix buffer volume	LYSIS buffer volume	WASH buffer volume	Recommended processing tube size		CRYO#20 volume
				First wash	All consequent washes	
100 µl	1.0 ml	4.0 ml	4.0 ml	10 – 15 ml	5-15 ml	100 µl
250 µl	2.5 ml	10 ml	10 ml	15 ml	15 ml	100 µl
500 µl	5.0 ml	20.0 ml	20.0 ml †	50 ml *	15 ml	250 µl
1.0 ml	10.0 ml	39.0 ml	40.0 ml †	50 ml *	15 ml	500 µl

* To achieve optimal cell yields execute only first spin after Fix&Lyse step in 50 ml tube, aspirate to mark 5 ml or above (not below 5 ml mark), transfer to 15 ml tube and continue with altered WASH buffer volumes.

† In case you decide to run all consequent processing steps in 15 ml tube, use 14 ml of WASH buffer and adjust number of washing steps based on pellet color. Typically, only one more washing step is required.

Guide on decision of RBCs lysis step duration:

Time after addition of LYSIS buffer						
	0 min	5-6 min	10 min	15 min	20 min	35 min
	No	No	No	OK	OK	OK

Conditions: Fresh whole blood fixed for 15 min at RT followed by addition of LYSIS buffer for indicated time

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