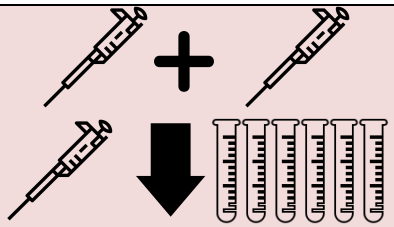
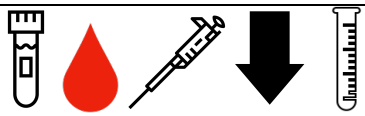
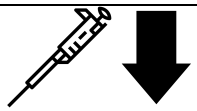
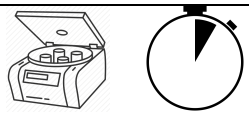
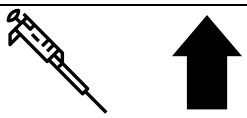
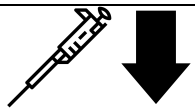
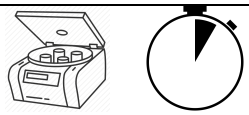
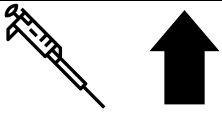
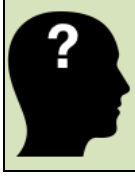
Processing after sample thawing

1. ☐		**Prepare 1x Fixation Buffer:** ↓ 1 part of 2x Fix-Concentrate ↓ 1 part of Fix-Diluent … and dispense to test tubes. Equilibrate at room temperature for 5 min.
2. ☐	 **37 ˚C**	**Thaw** blood samples cryogenically preserved in **Cytodelics Stabiliser** using 37 ˚C water bath (≈ 1 min).
3. ☐		Transfer **Stabiliser + blood** mixture to the test tube
4. ☐		**Incubate** sample at **+20˚C** for **15 minutes.** Vortex multiple times throughout.
5. ☐	**LYSIS** 	Add **LYSIS buffer** and vortex.
6. ☐		**Incubate** sample at **+20˚C** for 10-20 minutes (until the color of the solution is crystal clear red). **Do not proceed without complete RBCs lysis!**
7. ☐		Centrifuge sample at: **300g for 5-10 minutes**.
8. ☐		Aspirate supernatant but keep some volume at the bottom. **Do not decant!**
9. ☐	**WASH** 	Add **WASH buffer**.
10. ☐		Centrifuge sample at: **300g for 5-10 minutes**.
11. ☐		Aspirate supernatant but keep some volume at the bottom. **Do not decant!**
12. ☐	**#9, #10 & #11**	If the pellets contain remaining RBCs (pinkish- red), repeat washing procedure (WASH buffer/ centrifugation/supernatant aspiration).
13. ☐ 	☐ 13.a.	☐ 13.b.
		 **≤ -80 ˚C**
	Proceed to staining of sample.	Store samples in CRYO#20 and freeze at **≤ -80˚C**.

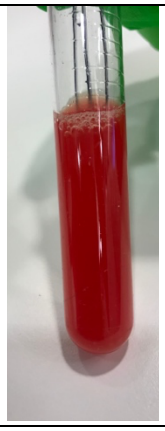
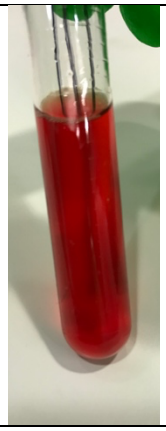
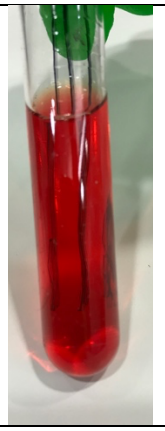
Scaling ratios		
Blood	1 : 1	Cytodelics Stabiliser
Blood	1 : 10	Fixation buffer
Blood	1 : 40	LYSIS buffer
Blood	1 : 40	WASH buffer
Blood	1 : 0.5 - 1	CRYO#20

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