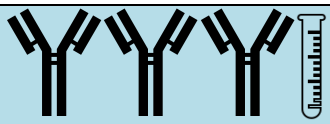
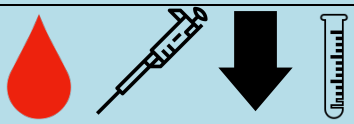
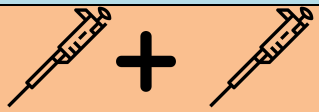
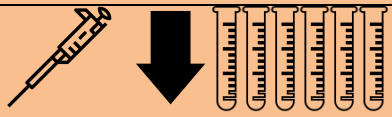
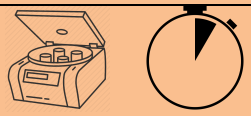
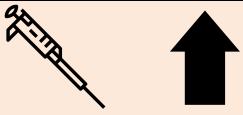
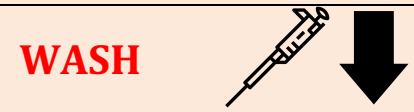
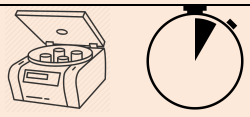
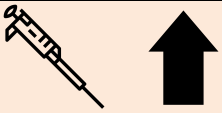
Whole blood processing kit
Graphical protocol
Staining of fixation sensitive epitopes

1. ☐		Prepare staining cocktail containing antibodies targeting sensitive epitopes
2. ☐		**Mix blood with the Ab-cocktail**
3. ☐		**Incubate at 5-8˚C/RT for 30-60 min**
		**Calculate required volume and prepare 1x Fixation Buffer 5-10 min before the end of the staining step:** ➧ 1 part of 2x Fix-Concentrate ➧ 1 part of Fix-Diluent Equilibrate at room temperature for 5 min.
4. ☐		**Dispense fixation buffer to tubes**
5. ☐		**Incubate** sample at **+20˚C** for **15 minutes.** Vortex multiple times throughout.
6. ☐		Add **LYSIS buffer** and vortex.
7. ☐		**Incubate** sample at **+20˚C** for 15-20 minutes (until the color of the solution is crystal clear red). **Do not proceed without complete RBCs lysis!**
8. ☐		Centrifuge sample at: **300g for 5 minutes**.
9. ☐		Aspirate supernatant but keep some volume at the bottom. **Do not decant!**
10. ☐		Add **WASH buffer**.
11. ☐		Centrifuge sample at: **300g for 5 minutes**.
12. ☐		Aspirate supernatant but keep some volume at the bottom. **Do not decant!**

13. ☐	**#10, #11 & #12**	If the pellets contain remaining RBCs (pinkish- red), repeat washing procedure (WASH buffer/ centrifugation/supernatant aspiration).	
14. ☐ 	☐ 14.a.	☐ 14.b.	
		**≤ -80 ˚C**	
	Proceed to staining of remaining epitopes/ICS.	Store samples in CRYO#20 and freeze at **≤ -80˚C**.	
15. ☐		 **37 ˚C** **Thaw** cryogenically preserved sample using 37 ˚C water bath (≈ 1 min)	
16. ☐		☐ **16.a.**	☐ **16.b.**
		Mass cytometry **Wash** once or twice with staining buffer	**Flow cytometry** **Acquire sample without washing or stain** directly in CRYO#20 (17.b.).
17. ☐		☐ **17.a.**	☐ **17.b.**
		 Follow your standard mass cytometry staining protocol	 Adjust volume by short spin 400g/2-3 min and aspirate supernatant. Leave 50-100 µl Proceed to staining of remaining epitopes/ICS.
18. ☐	ACQUIRE		

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.