

# Whole blood processing kit

## Sample stabilisation

### graphical protocol

Experiment ID:

Date:

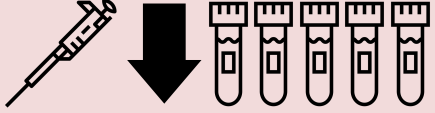
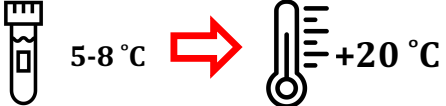










Operator:

#### Ratios of blood volume to kit components

	Volume	Recommended ratio to blood volume
Blood		
Stabiliser		1:1

#### Sample list

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1. <input type="checkbox"/>		Aliquot cryogenic vials with <b>Cytodelics Stabiliser (max. 1/3 of nominal volume)</b> . <i>Stored at 5-8 °C until sampling (up to 2 years).</i> <i>Identify dispensed volume on label.</i>	
2. <input type="checkbox"/>		Equilibrate at room temperature for 5 – 10 minutes.	
3. <input type="checkbox"/>		Transfer equal amount of blood to a cryogenic vial.	
4. <input type="checkbox"/>		Mix well by flipping the vial 10-15 times <b>DO NOT VORTEX!!!</b>	
5. <input type="checkbox"/>		Incubate at <b>+20 °C</b> for <b>min. 10 minutes</b> .	
6. <input type="checkbox"/>  	6.a. <input type="checkbox"/>	6.b. <input type="checkbox"/>	
	 $\leq -80\text{ °C}$	 $\leq -20\text{ °C}$	 <b>2M</b>
	 $\leq -80\text{ °C}$	 $\leq -80\text{ °C}$	 <b>1y</b>
	Store sample in ultra-low temperature freezer ( $\leq -80\text{ °C}$ ); keep until further processing (up to <b>1 year</b> ).	Store sample in freezer at $\leq -20\text{ °C}$ (up to <b>2 months</b> ). Transfer to ultra-low temperature freezer ( $\leq -80\text{ °C}$ ) as soon as possible and keep until further processing (up to <b>1 year</b> ).	

# Whole blood processing kit

## Processing after sample thawing

### graphical protocol

Experiment ID:

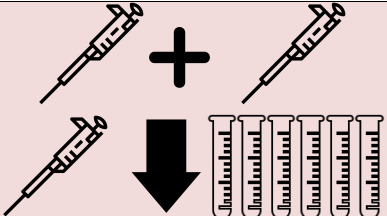





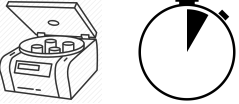
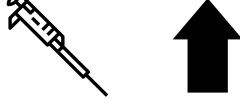

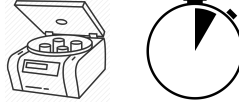
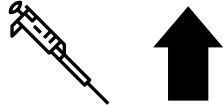




Date:

Operator:

Ratios of blood volume to kit components				
			Volume	Recommended volumes ratio blood : buffer
		Example	Your volume	
Blood/Stabilised blood		50 / 100 $\mu$ l		
Fix-Concentrate	Fix buffer	250 $\mu$ l	500 $\mu$ l	1:10
Fix-Diluent		250 $\mu$ l		
LYSIS buffer		2.0 ml		1:40
WASH buffer		2.0 ml		1:40

Sample list			
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1. <input type="checkbox"/>		<b>Prepare 1x Fixation Buffer:</b> ↓ 1 part of 2x Fix-Concentrate ↓ 1 part of Fix-Diluent ... and dispense to test tubes. Equilibrate at room temperature for 5 min.
2. <input type="checkbox"/>		<b>Thaw</b> blood samples cryogenically preserved in <b>Cytodelics Stabiliser</b> using 37 °C water bath (≈ 1 min).
3. <input type="checkbox"/>		Transfer 100 µL of the <b>Stabiliser + blood</b> mixture to the test tube
5. <input type="checkbox"/>		<b>Incubate</b> sample at +20°C for <b>15 minutes</b> . Vortex multiple times throughout.
6. <input type="checkbox"/>	<b>LYSIS</b> 	Add 2 mL of <b>LYSIS buffer</b> and vortex.
7. <input type="checkbox"/>		<b>Incubate</b> sample at +20°C for 10-20 minutes (until the color of the solution is crystal clear red). <b>Do not proceed without complete RBCs lysis!</b>
8. <input type="checkbox"/>		Centrifuge sample at: <b>400g for 5 minutes</b> .
9. <input type="checkbox"/>		Aspirate supernatant but keep some volume at the bottom. <b>Do not decant!</b>
10. <input type="checkbox"/>	<b>WASH</b> 	Add 2 ml of <b>WASH buffer</b> .
11. <input type="checkbox"/>		Centrifuge sample at: <b>400g for 5 minutes</b> .
12. <input type="checkbox"/>		Aspirate supernatant but keep some volume at the bottom. <b>Do not decant!</b>
13. <input type="checkbox"/>	 <b>#10, #11 &amp; #12</b>	If the pellets contain remaining RBCs (pinkish- red), repeat washing procedure (WASH buffer/ centrifugation/supernatant aspiration).
14. <input type="checkbox"/>	<input type="checkbox"/> 14.a.	<input type="checkbox"/> 14.b.
		 <b>≤ -80 °C</b>
	Proceed to staining of sample.	Store sample in freezer at ≤ -80°C. Use standard freezing media (e.g. 10% DMSO in FBS)