## Whole blood processing kit Graphical protocol Sample stabilisation

1.		Aliquot cryogenic vials with <b>Cytodelics</b> <b>Stabiliser (max. 1/3</b> of nominal volume). <b>Stored at 5-8 °C</b> until sampling (up to <b>2 years</b> ). Identify dispensed volume on label.
2.	5-8°C	C Equilibrate at room temperature for 5 – 10 minutes.
3.		Transfer equal amount of blood to a cryogenic vial.
4.		Mix well by flipping the vial 10-15 times DO NOT VORTEX!!!
5.		Incubate at <b>+20</b> °C for <b>min. 10 minutes</b> .
6.	6.a.	6.b.
?		₩≤ -20 °C 2M
	× 30°C ≥ •80°C	₩≤ -80 °C 1y
	Store sample in ultra-low	Store sample in freezer at $\leq -20^{\circ}C$ (up to <b>2 months</b> ) Transfer to ultra-low temperature
	(≤ -80°C); keep until further	freezer ( $\leq -80^{\circ}$ C) as soon as possible and keep
	processing (up to <b>1 year</b> ).	until further processing (up to <b>1 year</b> ).

Cytodelics AB, Stockholm, Sweden

Cytodelics

## **Processing after sample thawing**

1.	And the second s	<ul> <li>Prepare 1x Fixation Buffer:</li> <li>↓ 1 part of 2x Fix-Concentrate</li> <li>↓ 1 part of Fix-Diluent</li> <li> and dispense to test tubes. Equilibrate at room temperature for 5 min.</li> </ul>
2.	₩ 🗍 📫 🕼 37 °C	<b>Thaw</b> blood samples cryogenically preserved in <b>Cytodelics Stabiliser</b> using 37 °C water bath (≈ 1 min).
3.		Transfer 100 $\mu$ L of the <b>Stabiliser + blood</b> mixture to the test tube
5.		<b>Incubate</b> sample at <b>+20°C</b> for <b>15 minutes.</b> Vortex multiple times throughout.
6.	LYSIS	Add 2 mL of <b>LYSIS buffer</b> and vortex.
7.		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!
8.		Centrifuge sample at: <b>400g for 5 minutes</b> .
9.		Aspirate supernatant but keep some volume at the bottom. <b>Do not decant!</b>
10.	WASH	Add 2 ml of <b>WASH buffer</b> .
11.		Centrifuge sample at: <b>400g for 5 minutes</b> .
12.		Aspirate supernatant but keep some volume at the bottom. <b>Do not decant!</b>
13.	<b>()</b> #10, #11 & #12	If the pellets contain remaining RBCs (pinkish- red), repeat washing procedure (WASH buffer/ centrifugation/supernatant aspiration).
14.	14.a.	14.b.
?		°C \$\$\$
	Proceed to staining of sample.	Store samples in CRYO#20 and freeze at $\leq$ -80°C.

## Cytodelics AB, Stockholm, Sweden

Cytodelics