Standard Operation Procedure

Genomic DNA extraction from whole blood using Manta Onco

Kit contents

CONTENTS	QUANTITY (64 REACTIONS)	STORAGE
Proteinase K (lyophilized) (PK)	40 mg	-20°C (upon reconstitution)
Proteinase K Diluent (PKD)	2 mL	Room temperature
Blood Lysis Buffer (BL)	12.8 mL	Room temperature
Combs	8 nos	Room temperature
2 mL cartridges (pre-filled and sealed)	64 nos	Room temperature

Cartridge components (stored at room temperature)

WELL NUMBER	CONTENT	QUANTITY (PER REACTION)
1	Binding buffer	500 µL
2	Cambeads	200 µL
3	Wash buffer 1	500 µL
4	Wash buffer 2	500 µL
5	Wash buffer 3	300 µL
6	Elution buffer	100 µL

Items required by the user but not provided in the kit:

1. Manta Onco
2. Thermal shaker / Heat block

Preparation of working solutions

1. **Proteinase K solution**: Reconstitute the lyophilized **Proteinase K** powder by adding **2 mL** of Proteinase K diluent. After reconstitution, the Proteinase K is stored at -20°C.

Recommended sample volume for starting

A starting sample volume of 200 µL of whole blood is suggested for blood DNA extraction. Whole blood samples collected in K2-EDTA and K3-EDTA vacutainers, stored at 4°C, frozen, and at RT can be used for whole blood genomic DNA extraction.

Protocol

1. Pre-digestion of the blood sample

a) Add **30 µL of Proteinase K solution** to 1.5 mL microcentrifuge tubes. Add **200 µL of a whole blood sample** to the tubes and add **200 µL of Buffer BL** to the blood samples.

b) Vortex the tubes containing the samples for 40 seconds and incubate them at 70°C in a heat block for 10 minutes.

Note: This pre-digested lysate will be transferred to the well I of the cartridge.

2. Handling the cartridge

a) Carefully peel the seal off the top of the cartridge and transfer the pre-digested blood samples to **Well I** of the cartridge containing the binding buffer. Mix the contents of **Well I** thoroughly using a pipette.

b) Ensure that the cartridges fit in the deck tray properly. Place the filled cartridges onto the Manta deck tray.

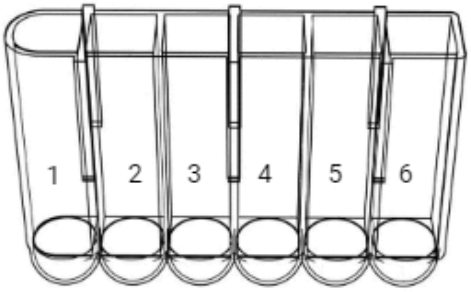

Fig 1 - Schematic representation of cartridge wells with sample and respective buffers
Well 1 - 430 µL Pre-digested blood lysate* + 500 µL Binding buffer
Well 2 - 200 µL Magnetic beads
Well 3 - 500 µL Wash buffer I
Well 4 - 500 µL Wash buffer II
Well 5 - 300 µL Wash buffer III
Well 6 - 100 µL Elution buffer

*The pre-digested blood lysate comprises of 30 µL Proteinase K + 200 µL whole blood + 200 µL Buffer BL

3. Set-up and run

a) Choose the **Open door** option on the main screen.

b) Remove the tray from the machine and place it in the bio-safety hood. Add 430 µL of lysate from step 1 to **Well I** of the cartridge.

c) Fit the magnetic sleeves on the machine, ensure a click to confirm loading. Place the tray into the machine. Ensure that cartridges are loaded properly.

d) Select the '**Choose extraction protocol**' option on the main screen.

e) Select the **'CB-200-i3'** option. Touch the ▶ icon and then select '**Continue'**.

f) After the extraction protocol is completed, collect the eluted DNA in a DNAse free microcentrifuge tube and store the elute at -20°C for long term storage.

g) Return to the main menu, and proceed with sterilisation protocol to ensure safety.