

# Standard Operation Procedure-CBWM068LB.64

## Genomic DNA Isolation from Whole Blood kit pre-filled cartridge for Manta (High Volume)

### Kit contents

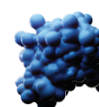
CONTENTS	QUANTITY (64 REACTIONS)	STORAGE
Proteinase K (lyophilized) (PK)	42 mg	4°C upon receipt & -20°C upon reconstitution
Proteinase K Diluent (PKD)	2.2 mL	Room temperature
Blood Lysis Buffer (BL)	42 mL	Room temperature
LE Buffer	4.4 mL	Room temperature
Combs	8 nos	Room temperature
2 mL cartridges (pre-filled and sealed)	64 nos	Room temperature
Elution buffer (for blanking purposes)	2 mL	Room temperature

### Cartridge components (stored at room temperature)

WELL NUMBER	CONTENT	QUANTITY (PER REACTION)
1	Binding buffer	500 µL
2	Cambeads	500 µL
3	Wash buffer 1	750 µL
4	Wash buffer 2	750 µL
5	Wash buffer 3	500 µL
6	Elution buffer	200 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 **2.1 mL** of Proteinase K diluent. After reconstitution, the Proteinase K is stored at  $-20^{\circ}\text{C}$ .

**Note:** Proteinase K is stable for at least 2 years at  $-20^{\circ}\text{C}$ . No loss of activity is observed after 10 freeze thaw cycles.

## Recommended sample volume for starting

A starting sample volume of 600  $\mu\text{L}$  of whole blood is suggested for blood DNA extraction. Whole blood samples collected in K2-EDTA and K3-EDTA vacutainers, stored at  $4^{\circ}\text{C}$ , frozen, and at RT can be used for whole blood genomic DNA extraction.

## Protocol

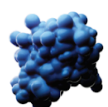
### 1. Pre-digestion of the blood sample

- a) Add **30  $\mu\text{L}$  of Proteinase K solution and 60  $\mu\text{L}$  LE Buffer** to 1.5 mL microcentrifuge tubes. Add **600  $\mu\text{L}$  of a whole blood sample and 600  $\mu\text{L}$  of Buffer BL** to the tubes in that order.
- b) Vortex the tubes containing the samples for 40 seconds and incubate them at  $70^{\circ}\text{C}$  in a heat block for 10 minutes.

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

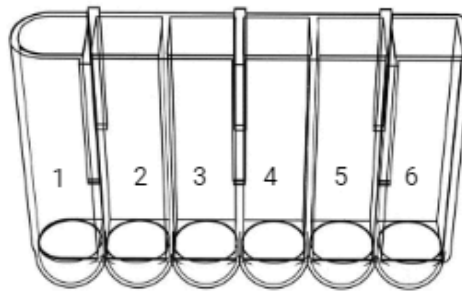
### 2. Handling the cartridge

- a) Gently Vortex and tap down the cartridge to make sure the contents of each well are settled at the bottom.
- b) Gently remove the seal from the top of the cartridge and transfer the **645  $\mu\text{L}$  of pre-digested blood lysate\*** and **10  $\mu\text{L}$  of CamBeads to Well I.**



Thoroughly mix the contents of Well I using a pipette.

- c) Transfer the **645  $\mu$ L of pre-digested blood lysate\* and 10 $\mu$ L of CamBeads to Well II**. Thoroughly mix the contents of Well II using a pipette. d) Vortex the bottle containing the beads after every 4 cartridges. e) Ensure that the cartridges fit in the deck tray properly. Place the filled cartridges onto the Manta deck tray.




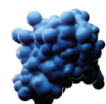
**Fig 1** - Schematic representation of cartridge wells with sample and respective buffers

- 1** - 645  $\mu$ L Pre-digested blood lysate\* and 500  $\mu$ L Binding buffer
- 2** - 645  $\mu$ L Pre-digested blood lysate\* and 500  $\mu$ L Binding buffer
- 3** - 750  $\mu$ L Wash buffer I
- 4** - 750  $\mu$ L Wash buffer II
- 5** - 500  $\mu$ L Wash buffer III
- 6** - 200  $\mu$ L Elution buffer

\*The pre-digested blood lysate comprises of 30  $\mu$ L Proteinase K, 60  $\mu$ L LE Buffer, 600  $\mu$ L whole blood and 600  $\mu$ L 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.
- 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-G**' 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 sterilization protocol to ensure safety.

