

Standard Operation Procedure-CBWM068.64

Genomic DNA extraction from whole blood using Manta

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)	14 mL	Room temperature
8 well 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)

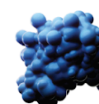
WELL NUMBER	CONTENT	QUANTITY (PER REACTION)
1	Binding buffer	500 µL
2	Cambeads	200 µL
3	Wash buffer 1	500 µL
4	Wash buffer 2	500 µL
5	Wash buffer 3	300 µL
6	Elution buffer	100 µL

Equipments required by the user:

1. Manta
2. Thermal shaker / Heat block

Preparation of working solutions

Proteinase K solution: Reconstitute the lyophilized **Proteinase K** powder by adding **2.1 mL of Proteinase K diluent**. After reconstitution, store the Proteinase K at -20°C.



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Note: Proteinase K is stable for at least 2 years at -20°C. No loss of activity is observed after 10 freeze thaw cycles.

Recommended sample volume for starting

A sample volume of 200 µL of whole blood is suggested for whole blood DNA extraction. Whole blood samples collected in K2-EDTA and K3-EDTA vacutainers, whether stored at 4°C, frozen, or at room temperature, are suitable for genomic DNA extraction.

Protocol

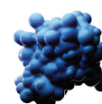
Pre-digestion of the blood sample

1. Add **30 µL of Proteinase K** solution to a 1.5 mL microcentrifuge tube. Then, add **200 µL of the whole blood sample**, followed by **200 µL of Buffer BL**.
2. Vortex the tube containing the sample 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.

Preparing the cartridges

1. Gently Vortex and tap down the cartridge to make sure the contents of each well are settled at the bottom.
2. Gently remove the seal from the top of the cartridge and Transfer the pre-digested **blood lysate* to Well I of the cartridge**, which contains the binding buffer. Thoroughly mix the contents of Well I using a pipette.
3. Ensure that the cartridges fit in the deck tray properly. Place the pre-filled cartridge onto the Manta deck tray.



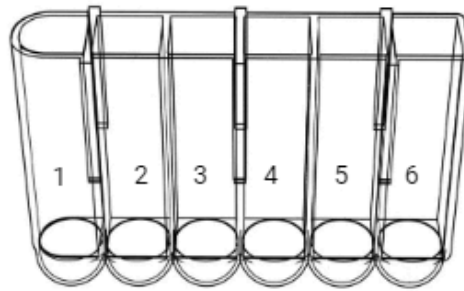



Fig. 1 - Schematic representation of cartridge wells with sample and respective buffers

- Well 1 - 430 μ L Pre-digested blood lysate* and 500 μ L Binding buffer
- Well 2 - 200 μ L Magnetic beads
- Well 3 - 500 μ L Wash buffer I
- Well 4 - 500 μ L Wash buffer II
- Well 5 - 300 μ L Wash buffer III
- Well 6 - 100 μ L Elution buffer

*The pre-digested blood lysate comprises 30 μ L Proteinase K , 200 μ L whole blood and 200 μ L Buffer BL.

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 μ L of lysate from step 1 to the **Well 1** 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 sterilization protocol to ensure safety.

