Standard Operation Procedure

Genomic DNA extraction from saliva 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
Saliva lysis buffer (SL)	26 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.

Before starting the preparation

Follow the instructions provided with the Cambrian saliva collection kit (**CBWA015**) to collect the saliva sample.

For best results, stabilize the samples for **at least 24 hours** at room temperature.

0.4 ml of stabilized saliva is recommended for optimal **gDNA extraction from saliva**.

NOTE

- *Ensure that the correct amount of starting material is used.*

Protocol

1. **Initial-lysis**

   - Add 400 µL of stabilized saliva and 30 µL proteinase K to the microcentrifuge tube containing the tissue sample. Vortex vigorously and ensure that the samples are completely submerged.
   - Incubate the microcentrifuge tube at **70°C for 15 min.**
   - transfer lysate to **Well 1** of the cartridge (containing the binding buffer).

2. Handling the cartridge

a) Carefully peel the seal off the top of the cartridge and transfer the pre-digested saliva samples to the **Well 1** of the cartridge containing the Binding buffer. Mix the contents of **Well 1** thoroughly using a pipette.

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

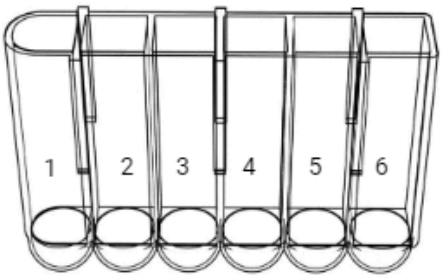

Fig 1 - Schematic representation of cartridge wells with sample and respective buffers

1 - 430 µL Pre-digested tissue lysate* + 500 µL Binding buffer

2 - 200 µL Magnetic beads

3 - 500 µL Wash buffer I

4 - 500 µL Wash buffer II

5 - 300 µL Wash buffer III

6 - 100 µL Elution buffer

*The pre-digested saliva lysate comprises of 30 µL Proteinase K + 400 µL of stabilized saliva

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 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 sterilisation protocol to ensure safety.

Supporting Protocol for sample collected using the DNA Genotek saliva collection kit:

- Add 400 µL of stabilized saliva sample to a 1.5mL centrifuge tube; add 30 µL PK.
- Vortex the centrifuge tube for 20 sec.
- Incubate the tubes at 70°C for 15 min.
- Proceed with the steps outlined in 'Protocol for genomic DNA extraction from Stabilized Buccal Swab Samples'.
- Remove the tray from the machine and place it in the bio-safety hood. Add 430 µL of lysate from step 1 to the **Well 1** of the cartridge.

Supporting Protocol for sample collected using 3rd Party lysis free saliva collection kits:

- Collect 1 ml of the stabilized sample, centrifuge at 14000 rpm for 10 minutes.
- Remove supernatant and add 400 µL of SL Buffer and 30 µL PK solution.
- Vortex for 1 minute and incubate at 70°C for 15 minutes.
- Proceed with the steps outlined in 'Protocol for genomic DNA extraction from Stabilized Buccal Swab Samples'.
- Remove the tray from the machine and place it in the bio-safety hood. Add 430 µL of lysate from step 1 to the **Well 1** of the cartridge.