Standard Operation Procedure

Manual Genomic DNA extraction from Buccal Swabs

Kit contents

CONTENTS	QUANTITY (10 REACTIONS)	STORAGE
Proteinase K (lyophilized)	6 mg	-20°C (upon reconstitution)
Proteinase K Diluent (PKD)	0.5 mL	Room temperature
Lysis Buffer (SL)	4 mL	Room temperature
Lysis - Binding Buffer (LBB)	5 mL	Room Temperature
CamBeads (CB)	0.250 mL	Room temperature
Wash Buffer I concentrate (WB1)	4.42 mL	Room temperature
Wash Buffer II concentrate (WB2)	3 mL	Room temperature
Elution Buffer (EB)	1 mL	Room temperature

How to start

Items required by the user:

- Cambrian Bioworks 8 station magstand (CBWD002) or equivalent

Additional buffers to be prepared by the user:

- 80% Ethanol

Recommended Sample volume:

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

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

Preparation of working solutions

1. **Proteinase K solution**: Reconstitute the lyophilized **Proteinase K** powder by adding 0.300 mL of Proteinase K diluent. After reconstitution the Proteinase K must be stored at -20°C.
2. **Wash Buffer I**: To the Wash Buffer I concentrate, add 3.58 mL of 100% ethanol and shake the bottle thoroughly before use.
3. **Wash Buffer II**: To the Wash Buffer II concentrate, add 5 mL of 100% ethanol and shake the bottle thoroughly before use.

A. Protocol for genomic DNA extraction from Buccal Swabs

Pre Processing:

1. Vortex the buccal swab sample collection tube for at least 20 sec at high speed.
2. Remove the swab from the collection tube and discard.
3. Aliquot **1 ml of the swab stabilization buffer** from the collection tube to a 1.5ml microcentrifuge tube; centrifuge the tube for **5min at top speed** (14,000 RPM)
4. Discard supernatant from the tube, add **400ul Lysis buffer (SL)** and **30ul proteinase K**
5. Vortex until the pellet is resuspended. Incubate the tubes at **70°C for 15 min**
6. Add **500 µL of Lysis - Binding Buffer** to the lysate and 20 µL of CamBeads to the microcentrifuge tube. Vortex the tubes for 15 minutes.
7. Place the tubes on the magstand. **Wait for 1 - 2 minutes** for the beads to settle on the wall of the microcentrifuge tube. Discard the supernatant.
8. Add **500 µL of Wash Buffer I** to the beads and remove the tubes from the stand. **Vortex the tube for 15 seconds** to wash the beads and place the microcentrifuge tube back on the stand.
9. Once the beads have settled against the wall of the microcentrifuge tube, **discard the supernatant**.
10. Add **500 µL of Wash Buffer II** to the beads and remove the tubes from the stand. **Vortex the tube for 30 seconds** to wash the beads and place the microcentrifuge tube back on the stand.
11. Once the beads have settled against the wall of the microcentrifuge tube, **discard the supernatant.**
12. Add **300 µL of 80% Ethanol** to the beads and remove the tubes from the stand. **Vortex the tube for 30 seconds** to wash the beads and place the microcentrifuge tube back on the stand.
13. Once the beads have settled against the wall of the microcentrifuge tube, **discard the supernatant.** Air dry the beads at 37°C for 3 minutes.
14. Remove the tube from the magstand. When all traces of ethanol are gone and the beads are dry, add **50 µL of pre-heated (pre-heated at 70°C for 2 min) elution buffer** to the tube.
15. **Vortex the tube for 5 minutes** for efficient elution and transfer the tube back to the magstand. Once the beads have settled against the wall of the microcentrifuge tube, collect the eluted DNA in a fresh DNAse- RNAse- free microcentrifuge tube and store it at -20°C.