Standard Operation Procedure

Genomic DNA extraction from stabilized saliva using CamBeads

Kit Contents

CONTENTS	QUANTITY (10 REACTIONS)	STORAGE
Proteinase K (lyophilized)	6 mg	-20°C (upon reconstitution)
Proteinase K diluent	0.5 mL	Room temperature
Buffer PL 3	4 mL	Room temperature
Lysis-Binding Buffer	5 mL	Room Temperature
CamBeads	0.250 mL	Room temperature
Wash Buffer I concentrate	4.42 mL	Room temperature
Wash Buffer II concentrate	3 mL	Room temperature
Elution Buffer	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 saliva collection kit (**CBWA015**) to collect the saliva sample.

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

1ml of stabilized saliva is recommended for optimal **gDNA extraction from saliva**.

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 is 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 Stabilized Saliva

Pre Processing:

1. Vortex the stabilized saliva sample for at least 20 sec at high speed. Add **1 ml stabilized saliva** to the 1.5ml microcentrifuge tube; centrifuge the tube for **5min at top speed** (14,000 RPM)
2. Remove supernatant from the tube, add **400 µl buffer PL3** and **30 µl proteinase K**
3. Vortex until the pellet is resuspended. Incubate the tubes at **70°C for 15 min**
4. 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.
5. Place the tubes on a magstand. Wait for 1-2 minutes for the beads to settle on the wall of the microcentrifuge tube. Discard the supernatant.
6. 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.
7. Once the beads have settled against the wall of the microcentrifuge tube, discard the supernatant.
8. 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.
9. Once the beads have settled against the wall of the magnet, discard the supernatant.
10. 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.
11. Once the beads have settled against the wall of the magnet, discard the supernatant. Air dry the beads at 37°C for 3 minutes.
12. 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.
13. 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 magnet, collect the eluted DNA in a fresh DNAse- RNAse- free microcentrifuge tube and store it at -20°C.