Standard Operation Procedure

GENOMIC DNA EXTRACTION FROM SALIVA USING ZYBIO EXM-3000

Storage condition:

The gDNA extraction kit (**CBWC085**) may be stored at room temperature.

Kit contents:

CONTENT	QUANTITY	STORAGE
Lysis Buffer	20 mL	Room temperature
Binding Buffer	25 mL	Room temperature
CamBeads (CB)	10 mL	Room temperature
Proteinase K (lyophilized) (PK)	30 mg	-20°C (upon reconstitution)
Proteinase K Diluent (PKD)	2 mL	Room temperature
Wash Buffer I (WB1)	25 mL	Room temperature
Wash Buffer II (WB2)	25 mL	Room temperature
Wash Buffer III (WB3)	15 mL	Room temperature
Elution Buffer (EB)	1.6 mL	Room temperature

How to start:

Items required by the user but not provided in the kit:

Equipment:

1. Zybio EXM-3000
2. 1.5ml centrifuge tubes

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.

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

Preparation of working solutions

1. Proteinase-K preparation

Reconstitute **30 mg** of lyophilized **Proteinase K** powder by adding **1.5 mL of Proteinase K diluent**. Vortex for 30 sec before use. After reconstitution, the Proteinase K solution ***must*** be stored at **-20°C**.

2. Buffer solution preparation

Buffers	**100% Ethanol to be added**	**Total Volume**
Wash Buffer 1	11.2 ml	25 mL
Wash Buffer 2	15.75 ml	25 mL
Wash Buffer 3	12 ml	15 mL

3. Vortex the CamBeads at high speed to ensure they are entirely dispersed

Pre Processing:

a. Vortex the sample for 20 sec. Take 1ml stabilized saliva and add it to the 1.5ml centrifuge tube, centrifuge the tube for 5min at top speed(14,000 RPM).
b. Remove supernatant from the tube, add 400ul lysis buffer and 30ul proteinase K.
c. Vortex until the pellet is resuspended. Incubate the tubes at 56°C for 20 min.

gDNA extraction from saliva - Plate description

Buffer	Volume	Well Position
Binding Buffer	500µL	Column 1 and 7
Lysate	400 µL	Column 1 and 7
CamBeads	200uL	Column 2 and 8
Wash Buffer I	500uL	Column 3 and 9
Wash Buffer II	5 00uL	Column 4 and 10
Wash Buffer III	300uL	Column 5 and 11
Elution buffer	100uL	Column 6 and 12

Note: *The volumes indicated above are per well.*

Protocol:

Plating saliva sample

a. Add the pre-processed sample to the plate (column 1 and 7)

Note: *For best results, use samples that have been stabilized for at least 24 hours or more.*

.Automated extraction procedure

1. Place the filled plates onto the Zybio platform and affix the magnetic sleeves properly. Select protocol "***Zybio-FFPE-Ver2***" and run the protocol.
2. After the protocol is run, collect the eluated DNA in a DNAse, RNase- free microcentrifuge tubes and store the eluted DNA at -20°C.

Troubleshooting

1. **The yields of the gDNA extraction are not as expected**

   Possible causes:

   a. The sample used was not collected as per the instructions provided with the Cambrian Bioworks saliva collection kit.
   b. The volume of sample used during the lysis / pre-treatment step was too low
   c. The protocol specified for the lysis / pre-treatment step was not followed correctly

2. The purity of the eluted DNA is not as expected

Possible causes:

a. The sample was not stabilized for the duration as specified in the lysis / pre-treatment protocol

3. Large variance in the yield of gDNA observed across the plate

Possible causes:

a. The Beads that are pre-filled in the plate may need to be resuspended. Shake the sealed plate thoroughly before use.

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

a. Add 400uL stabilized saliva to a 1.5mL centrifuge tube; add 30ul pK.
b. Vortex the centrifuge tube for 20 sec.
c. Incubate the tubes at 56°C for 20 min.
d. Transfer the lysate to the plate (Column 1 and 7)
e. Proceed with the steps outlined in the *'Automated extraction procedure*'