

# 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:

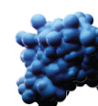
#### Equipment:

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**.

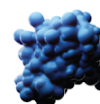
### 2. Buffer solution preparation

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:

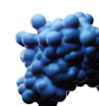
#### Plating saliva sample

- 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 Zybion platform and affix the magnetic sleeves properly. Select protocol "**Zybio-FFPE-Ver2**" and run the protocol.
2. After the protocol is run, collect the eluted 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*'

