Saliva DNA Extraction Protocol

Important Notes:

- Wear gloves and lab coat when handling the buffers provided in this kit. They contain irritants.
- Do not add acidic solution or bleach directly to the sample preparation waste.
- The precipitates that appear during storage, especially at low temperature, in some buffers are easily dissolved by incubating the bottles at 60°C.
- Store Proteinase K at -20°C.
- Add 20ml absolute ethanol to Wash Buffer I and 40ml to Wash Buffer II.

* Set thermoblock or water bath at 65°C.
* Preheat Elution Buffer at 65°C.
* Make sure that ethanol has been added into wash buffers.

1- Centrifuge about 1ml of saliva at 10000rpm for 2 min. Discard supernatant. If necessary, you can wash the sediment once with PBS.

2- Add 200µl **Binding Buffer** and 10µl **Proteinase K** to the sediment then vortex and spin briefly. Incubate at 65°C for 15 min.

3- Add 200µl absolute ethanol to the sample, vortex and spin briefly.

4- Transfer the mixture into the spin column placed in a collection tube.

5- Centrifuge for 1 min at 10000 rpm.

6- Discard collection tube. Place the column into a new collection tube.

7- Add 500µl **Wash Buffer I**, centrifuge for 1 min at 10000 rpm. Discard flow-through.

8- Place the column in a new collection tube, add 500µl **Wash Buffer II**, centrifuge for 1 min at 10000 rpm. Then proceed centrifugation at full speed (14000 rpm) for 2-3 min to dry the membrane completely.

9- Place the column in a clean 1.5ml microtube. Open the lid of the column and apply 50-100µl preheated **Elution Buffer** to the center of the membrane.

10- Close the lid and incubate at 65° C for 2-5 min. Centrifuge at full speed (>12000 rpm) for 1 min.

11- Store eluted DNA at -20°C.