dNEAT™ Saliva DNA Extraction Mini Spin Kit

PURK-SAL-050

Description

This Kit is a simple and rapid method for purification of high-quality DNA from human and animal sample saliva. The kit is based in DNA ability to bind silica in the presence of high concentrations of chaotropic salts.

Features

- ✓ Safe: no phenol-chloroform extraction.
- ✓ Efficient: Dependant on the sample material amount and type. 3-12 µg. Note that average DNA yield will vary depending on the donor (for example, health status).
- ✓ Ready to use genomic DNA, for all molecular biology applications.

Applications

gDNA purified with the kit is suitable for direct use in all common molecular biology applications: PCR, cloning, DNA sequencing, Southern blot analysis, etc.

Quality Certifications

This Kit is tested on a lot-to-lot basis by isolating total DNA from human sample saliva. DNA purified is analysed by spectrophotometer (Ratio 260/ 280 (1.6-1.8)) and agarose gel electrophoresis

Kit Components	
Minispin columns	50
Collection tubes (2 ml)	100
1XPBS	100 ml
BLY Buffer	20 ml
Proteinase K*	15 mg
WB1 Buffer	30 ml
WB2 Buffer**	6 ml
EB Buffer	10 ml

Storage

Store the kit at room temperature. If any kit reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves, and allow to cool to room temperature before use.

*Dissolve Proteinase K in water (1.5 ml) to obtain a 20 mg/mL stock solution. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed. This product as supplied is stable at room temperature.

**Add the volume ethanol (96%-100%) specified [Not included] to WB2 Buffer prior to initial use (see the label on the bottle for a volume indication). After ethanol has been added, mark the bottle to indicate that this step has been completed.

Product use limitation

This product is developed and sold exclusively for research purposes and use only. The product is not intended for diagnostics or drug development, nor is it suitable for administration to humans or animals.

Protocol

1. Collect ≈1 ml saliva by spitting in a 2 ml microcentrifuge tube (not provided). Try not to drag nasopharyngeal secretions. Avoid touching the mouth of the tube with the hands.
   - Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection. Homogenize the sample by shaking the tube containing the collected saliva (it is important to observe a homogeneous solution) and pipette 1 ml of the mixture to a new 2 ml microcentrifuge tube (not provided). The remainder of the saliva sample can be stored at room temperature until ready for further use.
2. Add 1 ml cold 1XPBS to the sample and mix well.
3. Centrifuge at full speed for 2 minutes to pellet the cells.
4. Carefully decant the supernatant and resuspend the pellet in 180 µl 1XPBS.
   - Vortex vigorously until the cells are resuspended (10-15 sec). This process will help to optimize the cell lysis in the following step.
5. [Optional Step] RNA Degradation: If RNA-free gDNA is required, add 4 µl of RNase A (100 mg/ml) (not provided).
6. Add 20µL proteinase K and 200 µL of buffer BLY and mix immediately by vortexing (it is important to observe a homogeneous solution).
7. Incubate in a water bath at 55 °C for 10 minutes.
8. Add 200 µl of ethanol (96–100%) and mix by vortexing vigorously.
9. Transfer the mix to the minispin column by pipetting and centrifuge at 8000rpm for 1 minute. Discard the flow-through solution.
10. Place the minispin column in a collection tube and add 500 µL of WB1 buffer. Centrifuge at 8000 rpm for 1 minute. Discard the flow-through solution.
11. Place the minispin column in a collection tube and add 500 µL of WB2 buffer. Centrifuge at 8000 rpm for 3 minutes. Discard the flow-through solution.
12. Centrifuge at full speed for 1 minute to dry the minispin column. This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
13. Place the minispin column into a new, labelled, 1.5 microcentrifuge tube (not provided) and pipet 50-100µL EB Buffer or pre-warm water directly into the membrane. Close the tube and incubate for 1 minute at room temperature.
14. Centrifuge at full speed for 1 minute to elute the DNA.
15. [Optional] You can repeat previous steps (13-14) for maximum yield.
16. The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.