TOOLS Buccal Swab and Saliva DNA Kit

For the purification of genomic DNA from a buccal or pharyngeal swab and saliva

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Introduction

The TOOLS Buccal Swab and Saliva DNA Kit uses a unique silica membrane technology and buffer system for the effective purification of gDNA (0.5–3.5 μg) from buccal or pharyngeal swabs and saliva. The aforementioned kit includes the Spin Column CB2, which consists of a new type of silica-gel membrane and can be easily bound by DNA. Polymerase chain reaction (PCR) inhibitors, such as divalent cations and proteins, are completely removed in two efficient wash steps. The remaining pure DNA is eluted using distilled water (pH 7.0–8.5) or a buffer provided with the kit. The genomic DNA isolated with the product is of high quality and can serve as an excellent template for various types of analyses, such as agarose gel analysis, restriction enzyme digestion, PCR analysis, and Southern hybridization analysis.

Kit Contents

Contents	DPT-BC62 (50 prep)s
Buffer GBHA	30 mL
Buffer GBBS	15 mL
Buffer RBD	24 mL
Buffer PBWE	25 mL
Buffer TB	15 mL
Proteinase K	500 μL
Number of spin columns (CB2)	50
Number of 1.5-mL collection tubes	50
Number of 2-mL collection tubes	50

Storage

The TOOLS Buccal Swab and Saliva DNA Kit can be stored in dry conditions at room temperature (15–25 °C) for up to 12 months without causing any reduction in its performance and quality.

Protocol

Ensure that ethanol (96%–100%) is added to Buffer GBBS (ethanol and Buffer GBBS have equal volumes), Buffer RBD (the volume of Buffer RBD is 1.5× that of ethanol), and Buffer PBWE (the volume of Buffer PBWE is 4× that of ethanol) before their use.

1. Sample preparation:
   a. To collect buccal cells, scrape the inside of the mouth 10 times with a buccal brush. Transfer the swab to a 2-mL microcentrifuge tube. Add 500 µL of GBHA and 10 µL of proteinase K to the tube, vortex the tube for 10 sec, and then incubate it at 65 °C for 30 min. Vortex the tube every 10 min. Collect 350 µL of the solution from the tube for the further DNA extraction steps.
   b. To collect pharyngeal swab cells, transfer the swab to a 2-mL microcentrifuge tube. Add 1 mL of GBHA (more GBHA can be purchased separately) to the tube, and mix by inverting the tube. Collect 350 µL of the solution from the tube and add 10 µL of proteinase K to it, vortex the mixture for 10 sec, and then incubate it at 65 °C for 30 min. Vortex the tube every 10 min.
   c. To obtain saliva DNA, add 300–500 µL of GBHA to an equal volume of saliva and mix by inverting the tube. Collect 350 µL of the solution from the tube and add 10 µL of proteinase K to it, vortex the tube for 10 sec, and then incubate it at 65 °C for 30 min. Vortex the tube every 10 min. If saliva has been preserved in other media, add half the volume of GBHA to the medium. Add 10 µL of proteinase K, vortex it for 10 sec, and then incubate it at 65 °C for 30 min. Vortex the tube every 10 min. Collect 350 µL of the solution from the tube for the further DNA extraction steps.
2. Add 500 µL of Buffer GBBS to the tube from step 1. Mix by inverting the tube, and incubate it at room temperature for 5 min.
3. Carefully transfer the entire lysate from step 2 to Spin Column CB2 (placed in a 2-mL collection tube), close the lid, and centrifuge the tube at 12 000 rpm (~13 400 × g) for 30 sec. Then, discard the filtrate and set Spin Column CB2 back into the 2-mL collection tube.
4. Add 500 µL of Buffer RBD (ensure that ethanol is added to Buffer RBD before use) to Spin Column CB2 and centrifuge it at 12 000 rpm (~13 400 × g) for 30 sec. Set Spin Column CB2 back into the 2-mL collection tube.
5. Repeat step 4.
6. Carefully open Spin Column CB2 and add 600 µL of Buffer PBWE (ensure that ethanol is added to Buffer PBWE before use). Close the lid and centrifuge the column at 12 000 rpm (~13 400 × g) for 30 sec. Then, discard the filtrate and set Spin Column CB2 back into the 2-mL collection tube.
7. Repeat step 6.
8. Set Spin Column CB2 back into the 2-mL collection tube and centrifuge the column at 12 000 rpm (~13 400 × g) for 2 min. Discard the filtrate and incubate Spin Column CB2 at room temperature (15–25 °C) for several minutes to dry the membrane completely.

   Note: This step is essential because ethanol carryover may interfere with downstream applications.

9. Place Spin Column CB2 in a clean 1.5-mL microcentrifuge tube, and pipette 50 µL of Buffer TB on the

center of the membrane. Close the lid and incubate the spin column at room temperature (15–25 °C) for 2–5 min. Finally, centrifuge the column at 12 000 rpm (~13 400 × g) for 2 min.

Note: The elution volume should not be less than 20 μL because a smaller volume will negatively affect the recovery efficiency.