EasyPrep Swab DNA Kit

For purification of genomic DNA from buccal swab

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Introduction

The EasyPrep Swab DNA Kit uses unique silica membrane technology and a buffer system for the efficient purification of gDNA. The Spin Column CR2 is made of a new type of silica-gel membrane that binds DNA specifically. PCR inhibitors, such as divalent cations and proteins, are removed in two efficient wash steps, leaving pure DNA to be eluted in either distilled water (pH 7.0–8.5) or a buffer provided with the kit. The isolated genomic DNA is of high quality and can serve as an excellent template for agarose gel analysis, restriction enzyme digestion, PCR analysis, and southern hybridization.

DNA yield

Sample	Sample Quantity	DNA Yeild
Buccal Swab	1	0.5-3.5 μg

Important Notes:

1. Check Buffer GBA and Buffer GBB before use for precipitation. Dissolve any precipitate by warming at 56 °C.
2. All centrifugation steps should be performed at room temperature (15–25 °C) in a standard centrifuge.

Kit Contents

Contents	DPT-BC22 (50 preps)	DPT-BC22-2 (200 preps)
Buffer GBA	30 ml	2× 50 ml
Buffer GBB	30 ml	2× 50 ml
Buffer GBD	13 ml	52 ml
Buffer PBW	15 ml	50 ml
Buffer TBB	15 ml	30 ml
Proteinase K	1 ml	4×1 ml
Spin Columns CR2	50	200
Collection Tubes（1.5 ml）	50	200
Collection Tubes（2 ml）	50	200

Reagents not supplied

RNaseA (100 mg/mL) and Carrier RNA

Storage

The EasyPrep Swab DNA Kit can be stored dry at room temperature (15–25 °C) for up to 12 months

without any reduction in performance or quality.

Protocol

Ensure that ethanol has been added to Buffer GBD and Buffer PBW as indicated on the tag before use.

To collect buccal cells, scrape the inside of the mouth 10 times with a buccal brush.

Note: To avoid contamination from food and beverage, do not eat or drink 30 min before sampling.

1. Dispense 400 µL of Buffer GBA into a 1.5-mL microcentrifuge tube. Remove the collection brush from its handle by using sterile scissors and place the detached head in the tube.

Note: If RNA-free DNA is required, 4 µL of RNaseA (100 mg/mL) can be added. Vortex for 15 s and incubate for 5 min at room temperature (15–25 °C).

2. Add 20 µL proteinase K, close the lid, and mix by pulse vortexing for 10 s. Incubate at 56 °C for 60 min. Vortex every 15 min.
3. Add 400 µL of Buffer GBB, close the lid, mix by pulse vortexing for 15 s, and incubate at 70 °C for 10 min. Briefly centrifuge the 2-mL microcentrifuge tube to collect liquid inside the lid. Squeeze to obtain as much lysate as possible and transfer the lysate into a clean 1.5-mL microcentrifuge tube.

Note: A white precipitate may form when Buffer GBB is added. The precipitate does not interfere with the procedure and will dissolve during incubation at 70 °C. If the precipitate does not dissolve, the cells have not lysed completely. This may result in a low yield of DNA and impure DNA. If less than 1 µg of genomic DNA is obtained, add an appropriate volume of dissolved carrier RNA to 400 µL of Buffer GBB.

4. Add 200 µL of ethanol (96%–100%), close the lid, and mix by pulse vortexing for 15 s. Briefly centrifuge the 1.5-mL microcentrifuge tube to collect liquid in the lid.

Note: Formed precipitates do not influence DNA yield.

5. Carefully transfer the entire lysate except the swab head from step 4 to the Spin Column CR2 (placed in a 2-mL collection tube), close the lid, and centrifuge at 12,000 rpm (~13,400 × *g*) for 30 s. Discard the filtrate and place the Spin Column CR2 in a 2 mL collection tube.
6. Carefully open the Spin Column CR2 and add 500 µL of Buffer GBD (ensure that ethanol has been added to Buffer GBD before use). Close the lid and centrifuge at 12,000 rpm (~13,400 × *g*) for 30 s, discard the filtrate, and place the Spin Column CR2 in a 2-mL collection tube.
7. Carefully open Spin Column CR2 and add 700 µL of Buffer PBW (ensure that ethanol is added to the Buffer PBW before use). Close the lid and centrifuge at 12,000 rpm (~13,400 × *g*) for 30 s. Discard the filtrate and place the Spin Column CR2 in a 2-mL collection tube.
8. Add 500 µL of Buffer PBW to the Spin Column CR2. Close the lid and centrifuge at 12,000 rpm

(~13,400 × *g*) for 30 s. Discard the filtrate.

9. Place the Spin Column CR2 back in a 2-mL collection tube and centrifuge at 12,000 rpm (~13,400 × *g*) for 2 min. Discard the filtrate and incubate the Spin Column CR2 at room temperature (15–25 °C) for several minutes to dry the membrane completely.

Note: This step is necessary because ethanol carryover into the eluate may interfere with downstream applications.

10. Place the Spin Column CR2 in a clean 1.5-mL microcentrifuge tube and pipet 20–50 µL of Buffer TBB onto the center of the membrane. Close the lid and incubate at room temperature (15–25 °C) for 2–5 min. Centrifuge at full speed (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 affect recovery efficiency.
For a high yield of DNA, the filtrate containing DNA can be added to the CR2 again, incubated at room temperature (15–25 °C) for 2 min, and centrifuged at 12,000 rpm (~13,400 × g) for 2 min.
The pH of the elution buffer influences elution efficiency. We recommend using Buffer TBB or distilled water (pH 7.0–8.5) to elute gDNA. For long-term DNA storage, elution in Buffer TBB and storage at −20 °C is recommended. DNA stored in water is subject to acid hydrolysis.

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