Ver. 24081

ODP314

Swab DNA Kit

For extraction of genomic DNA from buccal swab, throat swab, mouth wash, etc

Origin®

ISO 13485:2016 ISO 9001:2015

Swab DNA Kit

Spin Column
(ODP314)

Kit Contents

Contents	50 Preps
Buffer GA	10mL
Buffer GB	10mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Proteinase K (20mg/mL)	1mL
Carrier RNA	500μL
Spin column CB3	50
Collection Tubes (2mL)	50
Handbook	1

Storage

Swab DNA Kit could be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer term storage, the kit could be stored at 2-8°C.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary)

Proteinase K and carrier RNA should be stored at -20°C.

Introduction

Swab DNA Kit is based on silica membrane technology and special buffer system for many kinds of sample's genomic DNA extraction. The spin column made of new type silica membrane can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Extracted DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA extracted by Swab DNA Kit is suited for restriction analysis, PCR analysis, Southern blotting, and cDNA library.

Important Notes

1. Freezing and thawing of the samples to be avoided.
2. All centrifugation steps are carried out in conventional tabletop microcentrifuge at room temperature.
3. Increasing the time of absorption and elution could improve recovery efficiency.
4. Add appropriate amount of ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle before use.
   Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
5. If a precipitate has formed in Buffer GB1 or Buffer GD, warm buffer to 56°C until the precipitate has fully dissolved.
6. Repeated freezing and thawing of proteinase K should be avoided; otherwise it would reduce the DNA quality and quantity.

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Preparation of samples
   a) Buccal swab: Transfer the buccal swab sample into the 2 mL centrifuge tubes and add 500µL Buffer GA. Add 20µL Proteinase K, mix by vortex for 10 seconds, and incubate at 65°C for 15-30 minutes. Mix every 10 minutes by vortex. Pipette out 300-350µL solution to a fresh microcentrifuge tube and proceed with step 2.
   b) Pharyngeal swab: Transfer the pharyngeal swab sample into the 5mL centrifuge tubes and add 1-2mL Buffer GA, mix by inversion. Before extraction, pipette out 300-350µL solution to a fresh microcentrifuge and add 20µL Proteinase K, mix by vortex for 10 seconds, incubate at 65°C for 15-30 minutes and mix every 10 minutes by vortex.
   c) Saliva sample: Take the saliva sample as required, add equal volume Buffer GA and mix by inversion. Add 20µL Proteinase K, mix thoroughly by vortexing for 10 seconds, incubate at 65°C for 15-30 minutes and mix every 10 minutes by vortex..
2. Add 200µL Buffer GB to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 minutes to yield a homogeneous solution. Briefly centrifuge the 1.5mL microcentrifuge tube to remove drops from the inside of the lid.
   Note: Precipitates are expected, but it will not interfere with the extraction.
3. Add 10µL of Carrier RNA and mix the contents by vortexing for 10 seconds.
   Note: Add carrier RNA after the mixture cools down to room temperature
4. Add 200µL ethanol (96-100%) to the sample, and mix thoroughly by vortexing for 20 seconds. Briefly centrifuge the 1.5mL microcentrifuge tube to remove drops from the inside of the lid.
   Note: Precipitate formed in the earlier step will be dissolved.

5. Pipette the mixture from step 4 into the spin column CB3 (in a 2 mL collection tube) and centrifuge at 12,000 rpm (~13,400 ×g) for 45 seconds. Discard flow-through and place the spin column into the collection tube.
6. Add 500µL Buffer GD to spin column CB3, and centrifuge at 12,000rpm (~13,400 ×g) for 30 seconds, then discard the flow-through and place the spin column into the collection tube.
   Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
7. Add 700µL Buffer PW to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
   Note: A second wash with 700µL Buffer PW is recommended, if the salts have not been completely removed from the spin column.
8. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes to dry the membrane completely.
   Note: The resident ethanol of Buffer PW may have some affect in downstream application.
9. Place the spin column CB3 in a new clean 1.5mL microcentrifuge tube, and pipette 50-200µL Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15–25°C) for 2-5 minutes, and then centrifuge for 2 minutes at 12,000 rpm (~13,400 × g).
   Note: To increase the DNA yield, introduce the eluted Buffer TE to the column and centrifuge for 2 minutes at 12,000 rpm.
   If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting, we suggest chose buffer TE or distilled water (pH 7.0 - 8.5) to elute genomic DNA. For long-term storage of DNA, eluting in Buffer TE and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis