Ver. 24031

ODP313

Micro DNA Kit

For extraction of genomic DNA from micro volumes of sample

Origin®

ISO 13485:2016 ISO 9001:2015

Micro DNA Kit

Spin Column
(ODP313)

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

Micro 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

Micro 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. Purified DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA extracted by Micro 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 table-top microcentrifuge at room temperature.
3. Increasing the time of absorption and elution could improve recovery efficiency.
4. If a precipitate has formed in Buffer GB or Buffer GD, warm buffer to 56°C until the precipitate has fully dissolved.
5. 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. Concentration of samples: Follow this procedure to concentrate the sample if it is diluted or has few DNA containing cells. Otherwise proceed directly to Step 2
   a) Centrifuge the sample according to the table

Starting volume	Centrifugation speed
≤ 1.5mL	12,000 rpm for 30 seconds
≥ 1.5mL	5000 rpm for 2 minutes

   b) Discard the supernatant and resuspend the pellet in PBS as mentioned in the table

Starting volume	PBS
≤ 1.5mL	200µL
≥ 1.5mL	3-10mL

2. Add 5 volumes of Buffer GA to 1 volume of body fluid and mix thoroughly by vortexing.
   For example: Add 200µL Buffer GA to 40µL body fluid.
   Note: If RNA-free genomic DNA is required, add 4µL RNase A (10mg/mL) (ORT405-01), mix by vortexing for 15 seconds, and incubate for 5 minutes at room temperature (15–25°C). This step is to be performed
3. Add 20µL Proteinase K, mix thoroughly by vortexing. at 56°C until the sample is completely lysed.
4. 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.
5. 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
6. 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.
7. Pipette the mixture from step 6 into the spin column CB3 (in a 2mL collection tube) and centrifuge at 12,000 rpm (~13,400 ×g) for 45 seconds. Discard the flow-through and place the spin column into the collection tube.

8. 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.
9. Add 700µL Buffer PW to spin column CB3, and centrifuge at 12,000rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
10. Repeat step 9.
11. 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.
12. 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.