Ver. 23091

Universal DNA Purification Kit

Spin Column
(ODP214)

Kit Contents

Contents	50 Preps
Buffer PN	30mL
Buffer PW	15mL
Buffer EB	15mL
Spin Columns & Collection Tubes	50
Handbook	1

Storage

Universal DNA Purification Kit can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C.
(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary.)

Introduction

Universal DNA Purification Kit is based on silica-membrane technology. The kit can be used for purification of DNA fragments from various reaction solutions (PCR reactions, enzymatic reaction etc) by removing contaminants of protein, other organic compound, salts and primers, etc. The recovery yield is more than 80% for 100 bp-10 kb DNA fragments. The binding capacity of column is 5µg DNA per column. Purified DNA by the kit can be directly used in applications such as restriction enzyme digestion, PCR amplification, sequencing, library screening, ligation and transformation and so on.

Important Notes

1. Add 60mL ethanol (96-100%) to Buffer PW before use.
2. Minimize exposure of gels to UV radiation to improve the quality of DNA isolated.
3. All centrifugation steps are carried out at 12,000 rpm in conventional tabletop microcentrifuge at room temperature.
4. Increasing the time of absorption and elution could improve recovery efficiency for 10kb DNA fragment.
5. The recovery efficiency is related to starting DNA quantity and elution volume. The less starting quantity or elution volume, the less recovery efficiency.

Protocol

Add 60mL ethanol (96-100%) to Buffer PW before use (check bottle label for volume). All centrifugation steps are carried out at 12,000 rpm in a conventional table-top microcentrifuge at room temperature (15–25°C).

1. Sample processing
    a. In case of gel extraction- Excise the DNA fragment from the agarose gel and transfer it into a clean 1.5mL tube. Weigh the gel slice and add **THREE** volumes of Buffer PN. Incubate it at 65°C for 10 minutes and add **ONE** volume of isopropanol
    Note: For >2% agarose gels, add SIX volumes of Buffer PN
    b. In case of PCR purification, mix **ONE** volume of PCR sample with **THREE** volumes of Buffer PN and **TWO** volumes of Isopropanol. (For eg; 50µL PCR sample + 150µL Buffer PN + 100µL Isopropanol).

    Note: If the purification efficiency is low when isolating from gels, check solution pH after agarose gel dissolved completely. If pH > 7.0, add 10-30µL of 3M Acetic acid (pH 5.2) to the gel solution until the pH adjusted to < 7.0.
2. Place a spin column in a 2mL collection tube
3. Add solution from Step 1 into spin column. Centrifuge at 7,000 rpm for 1 minute. Discard the flow through and re-place the spin column to 2mL collection tube.

    Note: To get high yield DNA add flow-through solution into spin column and repeat the step 3.
4. Add 750µL Buffer PW to the spin column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-though and re-place the spin column.
5. Repeat step 4.
6. Centrifuge at 12,000 rpm for 3 minutes to remove residues. Discard the collection tube and place the spin columns to a new 1.5mL microcentrifuge tube (not provided).
7. Add 30-50µL Buffer EB. Incubate the mixture for 1 minute at room temperature. Centrifuge at 12,000 rpm for 2 minutes and discard the spin column.
8. Alternatively, for increased DNA concentration, add the solution gained from step 8 to the center of membrane again, let the columns stand for 1 minute, and then centrifuge.

    Note: The volume of Buffer EB must be more than 30µL, or it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting, we suggest Buffer EB or distilled water (pH 7.0-8.5) to elute DNA. For long-term storage of DNA, eluting in Buffer EB and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis.