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BIO BASIC INC.

EZ-10 96 WELL SPIN COLUMN PCR* PRODUCTS PURIFICATION KIT

BS3652 and BS365

Version 4.3
ISO9001 Certified

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Table of Content

Introduction	p3
List of Components	p3
Features	p4
Centrifugation Based Procedure	p5
Vacuum Based Procedure	p6
Troubleshooting Guide	p7
Other Kits Available	p8

Introduction

This kit provides a simple, efficient and high throughput method for purification of PCR* products and DNA from enzymatic reactions. The kit utilizes silica-gel membranes which selectively absorb up to 10μg of DNA fragments in each well in the presence of specialized binding buffers. Nucleotides, oligos (<40-mer), enzymes, mineral oil and other impurities do not bind to the membranes and are washed away. The DNA fragments can then be eluted off the column in small volume and used directly in downstream applications.

Kit Content

Components	BS3652 2 Plates	BS365 5 Plates
Buffer B3	48 ml	2x48 ml
Wash Solution	2x35 ml	4x48 ml
Elution Buffer	12 ml	30 ml
EZ-10 96 Well Spin Column Plate	2	5
Deep Well Collection Plate	4	10
96 Well Storage Plate	2	5
Sealing film	8	20
Protocol	1	1

(A) Before use, add 12 ml of 96-100% of Isopropanol to 48 ml of Buffer B3, or 24ml of 96-100% of Isopropanol to 96 ml of Buffer B3. For other volumes of Buffer B3, simply add enough isopropanol to make a 1:4 ratio (volume of added isopropanol: volume of Buffer B3 = 1:4).

(B) Before use, add 140 ml of 96-100% of ethanol to 35 ml of Wash Solution, or 192ml of 96-100% of ethanol to 48 ml of Wash Solution. For other volumes of Wash Solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol: volume of Wash Solution = 4:1).

(C) Elution Buffer is 2mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water may be substituted, the resulting yields may be up to 20% lower.

Note: For centrifugation based method, there is a minimum height requirement of 75 mm for apparatus to hold the assembly of EZ-10 96 Well Spin Column Plate and Deep Well Collection Plate.

Note:

(1) If PCR* mixture contains non-specific amplified DNA fragments, PCR* product should be purified using agarose gel. In this case, DNA Gel Extraction Kit (BS353) is recommended.

(2) This kit is not capable of removing the template DNA or primers with chain length longer than 40-mer.

Application

(1) Recovery of PCR* products from PCR* reaction mixture.

(2) Recovery of DNA fragments from reaction solutions.

Features

√ Rapid and Economical: entire procedure takes about 30 minutes to complete and 96 samples can be purified in parallel.

√ High yields (60-80%): it is suitable to recover 100 bp-40 kb DNA fragments.

√ Efficient Removal of Contaminants: purified DNA can be used in any downstream applications such as sequencing, labeling, restriction enzymatic digestions, ligations or transformations.

√ Convenient and Environmentally Friendly. No phenol / chloroform extraction or ethanol precipitation required.

Procedures

Part 1. Centrifugation Based Procedures

1. Transfer PCR* reaction mixtures to a Deep Well Collection Plate and add 3 volumes of Buffer B3, seal the Deep Well Collection Plate using a Sealing Film, mix by inverting 5 times.

Note: please ensure isopropanol has been added to Buffer B3 prior.

2. Place an EZ-10 96 Well Spin Column Plate on top of a new Deep Well Collection Plate. Transfer the above mixture solutions to the EZ-10 96 Well Spin Column Plate, and let it stand at room temperature for 2 minutes. Centrifuge at 5,700 x g for 5 minutes with a rotor for microtube plates.
3. Discard the flow-through. Add 500 µl of Wash Solution to the EZ-10 96 Well Spin Column Plate and spin at 5,700 x g for 5 minutes.

Discard flow-through and place the 96 Well Spin Column Plate back to the same Deep Well Collection Plate.

4. Repeat step 3. (Optional: Repeat wash step one more time if needed). Discard flow-through and spin again at 5,700 x g for 5 minutes to remove residual Wash Solution.
5. Place the EZ-10 96 Well Spin Column Plate on top of a 96 Well Storage Plate (deep-well plate). Add 30-50µl of Elution Buffer onto the center part of the column; incubate at 50 ºC for 4 minutes. Centrifuge at 4,500 x g for 5 minutes.
6. PCR* products are ready for use or kept at -20 ºC.

Part 2. Vacuum Based Procedures

(For details, please refer to Vacuum Manifold Product Information SD5011)

1. Transfer PCR* reaction mixtures to a Deep Well Collection Plate and add 3 volumes of Buffer B3, seal the Deep Well Collection Plate using a Sealing Film, mix by inverting 5 times.

Note: please ensure isopropanol has been added to Buffer B3 prior.

2. Assemble the Vacuum Manifold: place a Waste Tray in the Base, cover it with the Base Cap, and then place an EZ-10 96 Well Spin Column Plate on top. Transfer the above mixture solutions to the EZ-10 96 Well Spin Column Plate, and let it stand at room temperature for 2 minutes. Apply vacuum until the solution has passed through.

3. Discard the flow-through. Add 500µl of Wash Solution to the EZ-10 96 Well Spin Column Plate. Assemble the Vacuum Manifold as described in Step 2, apply vacuum until buffer has passed through.
4. Repeat wash procedure in step 3 (Optional: Repeat wash step one more time if needed). After Wash Solution has been drawn through the column, apply maximum vacuum for additional 2 minutes to dry the membrane. If necessary, tap dry the bottom nozzle of EZ-10 96 Well Spin Column Plate on paper towel before elution step.
5. For elution, assemble the Vacuum Manifold. This time, place a 96 Well Storage Plate Holder in the Base, and then put the 96 Well Storage Plate on top. Cover with the Base Cap. Place the EZ-10 96 Well Spin Column Plate from step 4 on top securely. Mark the orientation appropriately.
6. To elute DNA, add 30-50 µl Elution Buffer onto the centre of each well of the EZ-10 96 Well Spin Column Plate; incubate at 50 ^{o}C for 2 minutes. Apply vacuum for 1 minute. Switch off vacuum and ventilate vacuum manifold slowly.
7. Tightly seal the 96 Well Storage Plate. PCR* products are ready for use or kept at -20 ^{o}C.

Troubleshooting

Low DNA yield:

a) DNA less than 100bp or greater than 30kb may lead to a low recovery of DNA. Prolong the standing time after adding mixture to the spin column.
b) It is extremely important to add the Elution Buffer to the center of the column. Pre-warming the Elution Buffer to 80 ^{o}C or after adding the Elution Buffer to the column, incubate the column at 55 ^{o}C to 60 ^{o}C for 3-5 minutes.
c) Make sure Binding Buffer I does not have a precipitation, and ethanol have been added to Wash Solution before use.

Storage

The kit is stable for 18 months at room temperature. For longer storage, keep all contents in cold place.

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

Other Kits Available

EZ-500 Spin Column Plasmid DNA MaxiPreps Kit
BS465 (10preps), BS466 (20preps)
EZ-10 Spin Column Plasmid DNA MiniPreps Kit
BS413 (50preps), BS614 (250preps)
EZ-10 Spin Column DNA Gel Extraction Kit
BS353 (50preps), BS654 (250preps)

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