BIO BASIC INC.

# EZ-10 96 WELL SPIN COLUMN PLATE DNA CLEANUP MINIPREPES KIT

## BS3692 and BS369

Version 4.0
ISO9001 Certified

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## Introduction

This kit provides a simple, efficient, and automated high throughput method for purification of DNA fragments from variable enzymatic reactions such as cDNA synthesis, ligation, restriction enzyme digestions, tailing, PCR*, alkaline phosphatase, nick translation, due terminators products from PCR** reaction mixture. It is also an ideal tool to desalt the solution of DNA as well as to remove residual organic solvents or unincorporated nucleotides or primers (<40-mer) from reaction mixture. The kit utilizes a column which adsorbs selectively up to 10 µg DNA fragments in the presence of specialized binding buffers. Nucleotides, enzymes, mineral oil and other impurities are washed away. DNA fragments can be eluted readily with Elution Buffer.

## Kit Contents

| **Components** | **BS3692**<br>**2 Plates** | **BS369**<br>**5 Plates** |
|---|---|---|
| Cleanup Solution | 60 ml | 2x75 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 140 ml of 96-100% of ethanol to 35 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).
(B) 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 750 mm for apparatus to hold the assembly of EZ-10 96 Well Spin Column Plate and Deep Well Collection Plate.

## Application

(1) Recovery of PCR* products from PCR* reaction mixture.
(2) DNA Cleanup from the enzymatic reactions.
(3) Removal of nucleotides and primers (<40mer).

## 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 DNA mixtures to a Deep Well Collection Plate and add 3 volumes of Cleanup Solution. Seal the Deep Well Collection Plate using a Sealing Film, mix by inverting 5 times.
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 30-50 µl 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 see Vacuum Manifold Product Information)

1. Transfer DNA mixtures to a Deep Well Collection Plate and add 3 volumes of Cleanup Solution, seal the Deep Well Collection Plate using a Sealing Film, mix by inverting 5 times.
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 ºC 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 ºC.

## 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 ºC or after adding the Elution Buffer to the column, incubate the column at 55 ºC to 60 ºC for 3-5 minutes.
c) Make sure Binding Buffer I does not have a precipitation, and ethanol has been added to Wash Solution before use.



## Storage

The kit is stable for 12 months at room temperature. For longer storage, keep all contents in cold place.

* The Polymerase Chain Reaction (PCR) is covered by patents owned by Hoffman-La Roche Inc.

**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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