



# **PRODUCT INFORMATION**

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

<b>Components</b>	<b>BS365 , 5 Plates</b>	<b>BS366 , 10 Plates</b>
Binding Buffer I	3x50 ml	6x50ml
Wash Solution	3x40ml	6x40ml
Elution Buffer	30ml	60ml
EZ-10 96-well-plate	5	10
Deep Well Collection Plate	10	20
96 Storage plate	5	10
Sealing film	20	40
Protocol	1	1

- (A) Before use, add 240ml of 96-100% of ethanol to 60ml 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.

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

Note: The purification method is based on centrifugation. There is a minimum height requirement of 750mm for apparatus to hold the assembly -- filter plate and deep well plate.

### **Principle**

This 96-well kit provides a simple, efficient and automated high throughput method for purification of PCR\* products and DNA from enzymatic reactions. EZ-10 96-well spin column purification kits utilize the silica-gel membranes which selectively absorb up to 10ug 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 in downstream applications without further processing.

### **Note:**

- (1) If PCR\* mixture contains non-specific amplified DNA fragments, PCR\* product should be purified by 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:**

- Recovery of PCR\* products from PCR\* reaction mixture.
- Recovery of DNA fragments from reaction solutions.

### **Features:**

- Rapid and economical. Entire procedure takes about 30 minutes to complete 96 samples purification..
- 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.
- No phenol / chloroform extraction or ethanol precipitation



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## **Procedure for Purification of PCR\* Products:**

1. Transfer PCR\* reaction mixtures to a Deep Well Collection Plate and add 3 volumes of Binding Buffer I, seal the Deep Well Collection Plate by sealing film, mix by inverting 5 times.
2. Place a 96-well spin column plate in the top of a new Deep Well Collection Plate. Transfer the above mixture solutions to the 96-well spin column plate, and let stand at room temperature for 2 minutes. Centrifuge at 4,000 rpm for 5 minutes with a rotor for microtube plates.
3. Discard flow-through. Add 500ul Wash Solution to the 96-well spin column plate and spin at 6,000rpm for 5 minutes. Discard flow-through and place the 96-well spin column plate back to the same Deep Well Collection Plate.
4. Add 500ul Wash Solution to the EZ-10 96-well plate, spin at 6,000 rpm for 5 minutes. Discard flow-through and spin once more for 15 minutes to remove residue of Wash Solution.
5. Place the 96-well spin column plate in the top of a 96-well storage plate (deep-well plate). Add 30-50ul Elution Buffer onto the center part of the column, incubate at 50°C for 4 minutes. Centrifuge at 8,000 rpm for 5 minutes.
6. PCR\* products are ready for use or kept at – 20°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°C or after adding the Elution Buffer to the column, incubate at 55°C to 60°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.

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