PRODUCT INFORMATION

Enzymax **Tel: 8592198482 Web: www.enzymax.net**

EZ96-201 96 well PCR Clean-up kit

Components	EZ96-201 , 2 Plates	EZ96-201 , 5 Plates
Binding Buffer I	60 ml	2 x 75 ml
Wash Solution	2 x 35 ml	4 x 48 ml
Elution Buffer	12 ml	30 ml
EZ96DBP plates (DNA Binding plates)	2	5
EZ96DWP plate (2-ml deep well collection plates)	4	10
96 Storage plate	2	5
Sealing film	8	20
Protocol	1	1

(A) Before use, add 140 ml of 96-100% of ethanol to each of 35 ml Wash Solution.
(B) Elution Buffer is 2 mM Tris-HCl, pH 8.0~8.5. TE buffer (pH 8.0) or water may be used instead.
Storage: The kit is stable for 18 months at room temperature. For longer storage, keep all contents cold.
Note: The purification method is based on centrifugation. Vacuum Manifold can also be used.

Principle

This 96-well kit provides a simple, efficient and automated high throughput method for purification of PCR products and DNA from enzymatic reactions. The 96-well PCR clean-up kit (EZ96-201) utilizes the silica membranes which selectively absorb up to 10 μg 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 membrane and are washed away. The DNA fragments can then be eluted off the well in small volume and used in downstream applications without further processing.

Note:

(1) If PCR mixture contains non-specifically amplified DNA fragments, PCR product should be purified by agarose gel.
(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 purification of 96 samples.
- 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 enzyme digestions, ligation or transformation.
- No phenol/chloroform extraction or ethanol precipitation.

Procedure for Purification of PCR Products:

1. Transfer PCR reaction mixtures to a EZ96DWP plate (Deep Well Plate) and add 3 volumes of Binding Buffer I, seal the Deep Well Plate by sealing film, mix by inverting 5 times.
2. Place an EZ96DBP plate (Binding Plate) on the top of a new Deep Well Plate. Transfer the above mixture solutions to the Binding Plate, and let stand at room temperature for 2 minutes. Centrifuge at 4,000 rpm for 5 minutes in a rotor for microtube plates.
3. Discard flow-through. Add 500 μl Wash Solution to the Binding Plate and spin at 6,000 rpm for 5 minutes. Discard flow-through and place the Binding Plate back to the same Deep Well Plate.
4. Add 500ul Wash Solution to the Binding 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.
   Place the Binding Plate on top of a 96-well storage plate (new deep-well plate can be used as well). Add 30-50 μl Elution Buffer onto the center part of the column on Binding Plate; incubate at 50°C for 4 minutes. Centrifuge at 8,000 rpm for 5 minutes. **NOTE:** 96-well Storage Plate is very fragile and needs to be placed on top of a Deep Well Plate for support during centrifugation.
5. PCR products are ready for use or kept at – 20°C.

Troubleshooting for low DNA yield:

a) DNA less than 100 bp or greater than 30 kb 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.