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EasySC PCR Clean-up Kit

Catalog Number: D102-1, D102-2

Table 1. Kit Components and Storage

Kit Component	D102-1 (50 preps)	D102-2 (200 preps)	Storage	Stability
Buffer PB	10 mL	30 mL	RT	The product is stable for one year when stored as directed.
Buffer PW*	15 mL	2×25 mL	RT	
Elution buffer	15 mL	25 mL	RT	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

***** Prior to use, add absolute ethanol to **Buffer PW** according to the bottle label.

Product Description

EasySC PCR Clean-up Kit is designed for the rapid purification of single or double-stranded DNA from PCR and other enzymatic reactions. The system follows a “bind-wash-elute” procedure and completely removes primers, nucleotides enzymes, salts, and other impurities from a DNA sample. This convenient spin-column format eliminates the need for expensive resins or toxic organic compounds such as phenol and chloroform, thereby making it possible to process multiple samples in parallel. Purified DNA can be used in T-A ligations, sequencing, restriction enzyme digestion, and various other labeling reactions.

Features

- Rapid – Purification of PCR products in less than 10 minutes.
- Safe – No Phenol/chloroform extractions.
- High-quality – DNA is suitable for a variety of downstream applications.

Purification Protocol

1. Perform agarose gel electrophoresis to analyze PCR product.
2. Centrifuge to collect the PCR product to the bottom of the tube. Determine the volume of your PCR reaction. Transfer the sample into a clean 1.5 mL microcentrifuge tube.
3. Add 2 volumes Buffer PB. For PCR products smaller than 200 bp, add 2 volumes Buffer PB and 0.4 volumes 100% isopropanol.

   Note: Buffer PB may be precipitated during storage, if happen, heat it at 37^{o}C to dissolve.
4. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.
5. Insert a DNA Mini Column into a 2 mL Collection Tube. Transfer the samples from step 4 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.
6. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer PW to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.

   Note: Buffer PW must be diluted with absolute ethanol according to the bottle label before use.
7. Repeat step 6 for a second wash step.

8. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

   Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.

9. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube, add 30-50 µL Elution Buffer directly to the center of column matrix. Let sit at RT for 2 min, then centrifuge at 10,000 x g for 1 min.

   Note: To improve the yield, repeat this step for a second elution step.

10. Discard the column and store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Low yield	• Insufficient amount of Buffer PB: measure the volume of PCR product correctly, then add 2 volumes of Buffer PB. For PCR products smaller than 200 bp, add another 0.4 volumes 100% isopropanol. • Insufficient elution buffer: increase the volume of elution buffer and increase elution step. • Buffer PW is not diluted with ethanol: Buffer PW must be diluted with absolute ethanol before use.
Poor performance in downstream applications	• Salt contamination: add buffer Buffer PW to the column, let sit at RT for 5 min, then centrifuge. • Ethanol contamination: after centrifuging the empty DNA Mini Column at 12,000 x g for 3 min, open cap and let sit at RT for 5-10 min to completely dry the membrane.