Gene-Foci Biotechnologies

PCR Purification Kit

- Catalog No. GF2707
- User's Manual
- For Research Use Only
- In vitro Use Only

PCR Purification Kit

Catalog No.：GF2707

Catalog No.	Preps
GF2707-50	50
GF2707-200	200

❖ APPLICATIONS

Ideal for purification of PCR product, enzyme digested DNA fragment and nick-translation method labeled DNA probe. Also good for concentration of diluted DNA sample.

❖ Kit Contents And Storage Conditions

PCR Purification Kit	Storage Conditions	50 preps	200 preps
Binding Buffer BB	Room Temp.	50 ml	200 ml
Wash Buffer WB	Room Temp.	15 ml Add ethanol before first use	60 ml
Elution Buffer EB	Room Temp.	10 ml	40 ml
Binding Column EC	Room Temp.	50	200
2 ml Tubes	Room Temp.	50	200

This kit can be stored at room temperature for up to 12 months without showing any decrease in quality and yield.

❖ NOTES

1. All buffers should be clear. Lower temperature may cause precipitation. If any precipitation forms, warm up at 37°C water bath to dissolve before use.
2. The Gene-Foci PCR purification kit should be stored at room temperature, store at 4°C or -20°C may cause chemical compound precipitation in buffers.
3. Recap the bottles immediately after use to avoid unexpected oxidation, evaporation and change of pH due to long term exposure to the air.

❖ INTRODUCTION

The Gene-Foci PCR purification kit utilizes the highest quality silica matrix to recover DNA fragments from 100bp to 40kb free of oligonucleotides, nucleotides, and polymerase in yields exceeding 80%-90%. The binding condition of the silica matrix is adjusted by addition of a specially formulated buffer before adding the sample. Following a rapid wash step, DNA is eluted with EDTA-free low salt and high pH elution buffer or double distilled water. Purified DNA can be directly used for most downstream applications including restriction enzyme digestion, ligation, Sanger sequencing, DNA labeling and in vitro transcription.

❖ HIGHLIGHTS

1. High quality silica membranes are used to ensure the yield and consistency between different batches.
2. Unique guanidine hydrochloride and sodium iodide-free binding buffer ensures high purity of DNA product.

3. Yellow pH indicator helps monitoring the pH change in solutions and maximizing DNA binding 1to silica matrix.

4. Fast and toxic-free. No phenol chloroform extraction, no ethanol precipitation **is required.**

❖ ATTENTION

1. All the steps are performed at room temperature, use microcentrifuge such as Eppendorf 5415C or similar model that can handle 13,000 rpm or higher speed.

2. Binding buffer BB and equilibrium buffer contain irritating chemicals, wear gloves when handling. **Avoid direct contact with skin, eyes and clothes. If contaminated, rinse with large amount of water immediately.**

3. The size of the DNA ideal for PCR purification kit is between 100bp and 10 kb, DNA yield drops sharply beyond this range.

4. DNA yield is correlated with the amount of DNA to begin with, elution volume and the size of DNA fragment.

5. Elution buffer EB does not contain chelator EDTA, thus minimizes the effect on downstream experiment such as enzyme digestion, ligation. Alternatively, the plasmid DNA can be eluted with water. However, to ensure efficient elution, the pH of the water must be equal or higher than 7.5. Plasmid DNA should be stored at -20℃.

❖ GENE-FOCI PCR PURIFICATION KIT PROTOCOL

Hints：

- ⇨ Before start, add the indicated amount of ethanol into buffer WB, mix well, and mark the bottle with a check.
- ⇨ For the DNA binding column EC that have been stored for more than 3 months, to restore binding capacity, pre-treat the column with equilibrium buffer (available form Gene-Foci.com) is recommended. Pre-treat the binding column EC by adding 100µl equilibrium buffer and centrifuging at 13,000rpm for 1 minute.
- ⇨ Before start, it is recommended to check the PCR product with agarose gel electrophoresis to make sure the PCR reaction is successful.

1. **For every 100µl PCR product, add 500µl binding buffer BB and mix well.** (If the PCR product volume is smaller than 100µl, adjust the volume to 100µl with ddH_2O).
2. **Place a binding column EC into a provided 2 ml collection tube, add the mixed sample from step 1 to the column, incubate at room temperature for 1 minute.**
3. **Centrifuge at 13,000 rpm for 30-60 seconds.**
4. **Discard the flow-through, place column EC back into the same tube.**
5. **Wash column EC by adding 0.7 ml buffer WB and centrifuging at 13,000rpm for 30 seconds.**
   Make sure ethanol has been added into buffer WB.
6. **Discard the flow-through, place column EC back into the same tube.**
7. **Repeat the wash in step 4 one more time with 0.5 ml buffer WB.**
8. **Discard Flow-through, put the binding column EC back into the same 2ml centrifuge tube, spin at 13,000rpm for additional 2 minutes to remove residual wash buffer.**
   Residual ethanol in buffer WB may inhibit subsequent enzymatic reactions such

as restriction enzyme digestion and ligation.

9. **Transfer the spin column AC into a clean 1.5 ml centrifuge tube.**

10. **To elute, add 50 µl elution buffer (buffer EB) to the center of the silica membrane in the column, (for better yield, pre-warm up buffer EB to 65-70°C in a water bath), incubate at room temperature for 2 minutes，and centrifuge at 12,000 rpm for 1 minute. For better yield, re-apply the eluate into the column, repeat the centrifugation.**

❖ TROUBLE SHOOTING

Problem	Possible causes and suggestions
Low yield	*Kit is stored at suboptimal temperature (for example,4°C or -20°C), chemicals in buffers precipitated. **-Suggestion:** Always store the PCR purification kit at room temperature. *Not enough binding buffer BB added to PCR product. **-Suggestion:** add 5x or 6x volumes of buffer BB to every 1x volume of PCR product. *PCR product and binding buffer BB did not mix well. **-Suggestion:** gently vortex or pipette up and down several times to mix well after adding buffer BB. *Forget to add ethanol into buffer WB **-Suggestion:** Make sure the appropriate amount of ethanol is added into buffer WB.
Poor elution	*Suboptimal elution buffer is used**-Suggestion:** Use buffer EB to elute DNA, do not use water.
DNA floats out of well while loading agarose gel	Ethanol in DNA sample. **-Suggestion:** Spin dry the columns as indicated in step 8 to get rid of ethanol from the binding column.
DNA is resistant to enzyme digestion	*Skipped step 9, ethanol in DNA eluate. **-Suggestion:** Spin dry the columns as indicated in step 8, then air dry the column for several minutes before elution. *Silica fines in eluate. **-Suggestion:** Spin the eluate at 13,000 rpm for 1 more minute, use the supernatant for enzyme digestion.

Ordering Information

To order Gene-Foci products, please try the following methods:

(1) Order online

Register for an account on www.Gene-Foci.com, login, and place your order using our shopping cart and secure online checking out system.

(2) Call our toll-free number +1-888-315-9018

(3) Send Email to order@Gene-Foci.com

(4) Fax your order to +1-888-959-0868

To expedite your order, please provide the following information:

Customer user name

Purchaser's name and detailed contact information

Purchase Order Number (If any)

Billing address

Shipping address

Description of the order