

QuickClean 5M PCR Purification Kit



Technical Manual No. 0182

Version 03252008

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I. DESCRIPTION

QuickClean 5M PCR Purification Kit is a reagent kit for fast DNA purification from PCR reactions and DNA cleanup from enzymatic reactions. Up to 20 µg DNA product (both double-stranded and single-stranded DNA, 70 bp-10 kb) can be recovered in 5 minutes from PCR reactions and enzymatic reactions. DNA with the size of 70 bp-10 kb is efficiently adsorbed on to the QuickClean column and other impurities such as enzymes or proteins, small DNA fragments or short oligonucleotides (short primers or probes), dyes, salts and nucleotides are washed out of the column. Eluted in a small volume low-salt buffer with complete removal of contaminants and inhibitors, the purified DNA is ready for most downstream applications such as PCR, transformation, restriction enzyme digestion, cloning, sequencing, *in vitro* translation, transfection, etc.

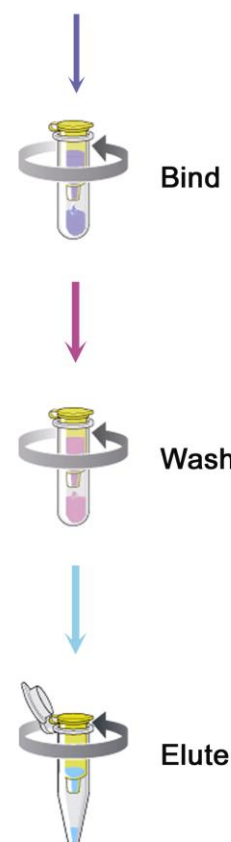
II. KIT CONTENTS

QuickClean 5M PCR Purification Kit contains the following reagents, mini-columns and collection tubes enough for 250 purifications.

| L00198 Components | 250 Preps |
|-----------------------|-----------|
| Binding solution I | 2 X 75 ml |
| Wash solution | 55 ml |
| Elution buffer | 25 ml |
| QuickClean columns | 250 |
| 2 ml Collection tubes | 250 |
| Protocol | 1 |

Overview of Kit Procedure

PCR or Enzymatic Reaction





III. APPLICATIONS

QuickClean 5M PCR Purification Kit enables the fast purification of high-quality DNA products from PCR reactions and enzymatic reactions ready for further downstream applications such as:

- PCR and cloning
- Restriction enzyme digestion
- Transformation
- Sequencing
- *in vitro* translation

IV. KEY FEATURES

- ◆ Easy to perform: simple and rapid procedure to purify DNA in 5 min.
- ◆ High capacity: columns with a capacity of 20 µg of DNA.
- ◆ High purity: with complete removal of contaminants and inhibitors.
- ◆ Reproducible yields: high (90-98% recovery) and reproducible yields of pure DNA every time.

V. STORAGE

This kit should be stored dry at room temperature. The kit is stable for 12 months at room temperature.

VI. PURIFICATION PROTOCOL

Before use, do the following:

1. Add 220 ml of 96-100% of ethanol to 55 ml of wash solution and mix well.
2. Binding solution may form some precipitate upon long storage. Gentle mixing can dissolve the precipitate, otherwise, dissolve the precipitate by warming the bottle at 37°C for a few minutes.

Purification procedure:

1. Transfer PCR reaction or enzymatic reaction to a 1.5 ml microcentrifuge tube. If the PCR reaction or enzymatic reaction tube is large enough, no transfer is necessary. Add 2.5 volumes of binding solution I and mix thoroughly by capping and inverting the tube several times. It is not necessary to remove mineral oil if there is any.
2. Transfer the above mixture to the column, and centrifuge at 12,000 rpm for 1 minute.
3. Discard the flow-through in the tube. Add 500 µl of wash solution to the column and centrifuge at 12,000 rpm for 30 seconds. If necessary, repeat wash procedure once.
4. Centrifuge at 12,000 rpm for an additional 1 minute to remove residual amount of wash solution.
5. Transfer the column to a clean 1.5 ml microcentrifuge tube. Add 30-50 µl of elution buffer to the center of the column membrane and incubate at room temperature for 1 minute. Centrifuge at 12,000 rpm for 1 minute to elute and collect DNA.

VII. EXAMPLES USING THE KIT



QuickClean 5M PCR Purification Kit was compared with a commercially available kit (competitor A) for rapid PCR DNA product purification from PCR reactions following the protocols provided by the manufacturers. The results were shown in Figure 1.

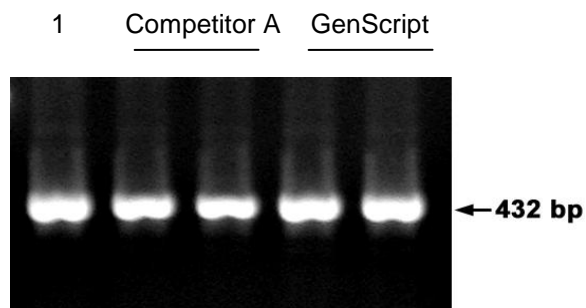


Figure 1. A kit from competitor A and QuickClean 5M Purification Kit were used for PCR DNA purification from PCR reactions following the protocols provided by the manufacturers. Two PCR DNA purifications were performed using each kit. The same volume of PCR reactions was used in each purifications. Lane 1 is before purification.

VIII. TROUBLESHOOTING

The table below is guideline for troubleshooting.

| Problem | Probable Cause | Solution |
|------------------------------|--|---|
| Low DNA recovery | Not enough binding solution was used. | Add 2.5 volumes of binding solution I to the reactions and mix thoroughly. |
| | The wash solution did not contain ethanol. | Before use, add 220 ml of 96-100% of ethanol to 55 ml of wash solution and mix well. Put a check mark in the box on the cap of the wash solution bottle. |
| | The reaction solution was highly basic. | In some rare cases, the reaction solution is highly basic. A proper volume of 3 M sodium acetate (pH 5.0 or lower) can be added to the solution to adjust the pH. The optimal binding pH is below 7). |
| | Other elution solution is used. | Elution buffer is 2.0 mM Tris-HCl pH 8.5. TE buffer (pH 8.0) or water can also be used, but yield may be slightly lower. |
| Small size nucleic acid band | Primer co-purification. | Primers longer than 50-mer are not completely removed. Short primers may form primer-dimers that cannot be completely removed, either. |

IX. ORDER INFORMATION



QuickClean 5M PCR Purification Kit:

Cat. No. L00198

For Research Use Only.

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