

QuickClean 96-Well PCR Purification Kit



Technical Manual No. 0220

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I. DESCRIPTION

The QuickClean 96-Well PCR Purification Kit is a reagent kit from GenScript. It contains all the necessary reagents, mini-columns, and collection tubes for the fast and reliable extraction of DNA from PCR reactions and for DNA cleanup in enzymatic reactions. In only five minutes, QuickClean can recover up to 20 µg of double-stranded DNA product. DNA molecules ranging in size from 70 to 10,000 base pairs are adsorbed onto the QuickClean Column while impurities such as enzymes and proteins, small DNA fragments, dyes, salts, nucleotides and short oligonucleotides (such as primers and probes) are washed away. Eluted in a small volume low-salt buffer, completely free of contaminants and inhibitors, the purified DNA is immediately ready for most downstream applications such as cloning, sequencing, and restriction enzyme digestion.

II. KIT COMPONENTS

Components	Size (4 x 96-well)
Binding solution	120 ml
Wash solution (concentrated)	2 x 50 ml
Elution solution	40 ml
96-Well binding plate	4
96-Deep-well plate	8
Well collection plate	4
Adhesive tape	8
Protocol	1

III. STORAGE

Store at room temperature.

IV. PURIFICATION PROTOCOL

Before use, perform the following:

1. Add 200 ml of 100% of ethanol to 50 ml of wash solution and mix well.
2. Some precipitate may form in the binding solution after long storage. Dissolve the precipitate by mixing gently or by warming the container to 37°C for a few minutes.

Purification procedure:

1. Transfer the PCR or enzymatic reactions to 1.5-ml microcentrifuge tubes. Add 2.5 volumes of binding solution I and mix thoroughly by capping and inverting the tubes several times. It is not necessary to remove any mineral oil.
2. Place a 96-well binding plate on the top of a 96-deep-well plate. Transfer the mixtures from step 1 to the 96-well binding plate.
3. Keep the plate at room temperature for two minutes.
4. Centrifuge at 2,000 rpm for one minute. Discard the flow-through in the 96-Deep-Well Plate and place the 96-Well Binding Plate back on the top of the same 96-Deep-Well Plate again.
5. Add 500 µl of wash solution to each well of the 96-well binding plate and seal the well securely with Adhesive Tape (supplied in the kit). Then centrifuge at 2,000 rpm for one minute. Discard the flow-through in the 96-Deep-Well Plate. If necessary, repeat the wash procedure one more time. Centrifuge at 2,000 rpm for an additional three minutes to remove any residual wash solution. Discard the 96-Deep-Well Plate.
6. Place the 96-well binding plate to the top of a 96-well collection plate. Add 30-50 µl of elution buffer to the center of the column membrane and incubate at room temperature for one minute.
7. Centrifuge at 4,500 rpm for two minutes to elute and collect DNA. Store the DNA samples at 4°C for short period (1-2 days) or at -20°C for long-term storage.

V. EXAMPLES

QuickClean 96-Well Purification PCR Kit is here used to purify DNA fragments of between 100 bp and 15 kb. The results are shown in figure 1.

M 1 2 3 4 5 6 7 8 9 10
U P U P U P U P U P U P U P U P

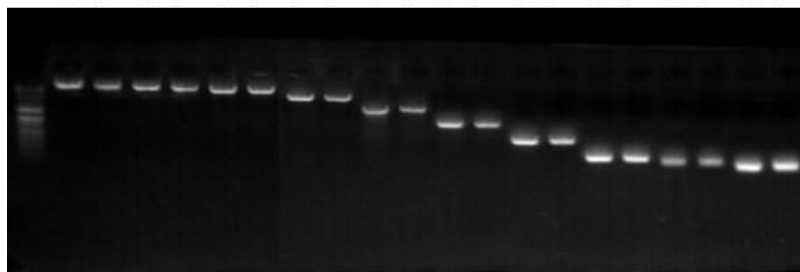


Fig. 1. PCR DNA purification using the kit.

M: DNA Logical Ladder (GenScript, M103R);
U: Unpurified PCR products;
P: Purified PCR products.

Lane 1: 15 kb
Lane 2: 10 kb
Lane 3: 8 kb
Lane 4: 4 kb
Lane 5: 2 kb

Lane 6: 1 kb
Lane 7: 500 bp
Lane 8: 200 bp
Lane 9: 150 bp
Lane 10: 100 bp

VI. 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 200 ml of 100% of ethanol to 50 ml of wash solution and mix well. Check the box on the cap of the wash solution bottle.
	The reaction solution is highly basic.	In 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.
	The elution was not properly performed.	After the addition of elution solution, incubate the plate at room temperature for at least one minute.
Small nucleic acid band	The primers have been co-purified.	Both very short primers that can dimerize and very long primers of over 50-units are not always completely removed.

VII. ORDERING INFORMATION

QuickClean 96-Well PCR Purification Kit: Cat. No. L00238

For Research Use Only.

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