High Pure PCR Cleanup Micro Kit

for purification of products from PCR and other reactions

Cat. No. 04 983 955 001 (up to 50 purifications)
Cat. No. 04 983 912 001 (up to 200 purifications)

Principle

Nucleic acids bind specifically to the surface of glass fibers in the presence of chaotropic salts. Since the binding process is specific for nucleic acid, the bound material can be separated and purified from impurities by a simple wash step. The Binding Enhancer enables the modification of DNA fragment size exclusions. Small oligonucleotides and dimerized primers from amplification reactions are selectively removed. The nucleic acids elute from the glass fiber fleece in a low-salt buffer or water.

Starting material

Samples (up to 100 µl each) could contain:

- Amplified DNA products that are between 50 bp and 5 kb long
- Modified DNA fragments [*e.g.*, DNA processed with restriction enzymes, T4 polymerase or other enzymes] that are between 50 bp and 5 kb long
- Hapten-labeled (*e.g.*, DIG-labeled) or fluorescently labeled DNA fragments
- RNA from *in vitro* transcription reactions
- First and second strand cDNA
- Samples (up to 100 mg) of agarose gel slices

Application

Use the High Pure PCR Cleanup Micro Kit to efficiently purify products from PCR and other reactions. The kit eliminates contaminants from amplification reactions (*e.g.*, primers, mineral oil, salts) and can also be used to purify nucleic acids from other modification reactions such as restriction endonuclease digests, alkaline phosphatase treatment, and kinase reactions. In addition, the kit can be utilized to purify cDNA, concentrate dilute nucleic acid solutions, and recover DNA from agarose gel slices. The purified DNA can be used directly in subsequent enzymatic reactions such as labeling, sequencing, cloning, and ligation, as well as for PCR analysis.

Time required

The entire High Pure PCR Cleanup Micro Kit method takes approx. 10 min.

Results

The amount of DNA recovered is dependent on the amount of DNA applied to the glass fiber fleece, the elution volume, and the length of the amplification/DNA products. When 5 - 25 mg DNA is applied to the kit's High Pure Micro Filter Tube, approximately 80% of the DNA can be recovered.

Benefits

- Conserve resources by using one versatile kit that eliminates the need to use several kits from other suppliers.
- Save time with a simple and rapid protocol that reduces purification time.
- Obtain purified product in a small elution volume ($\leq$10 µl) for demanding downstream applications.
- Efficiently remove contaminants and unwanted reaction components.
- Generate contaminant-free DNA for direct use in cloning, ligation, restriction digests, and other reactions.
- Selectively isolate specific DNA fragment sizes by using the kit's binding enhancer to adjust purification stringency

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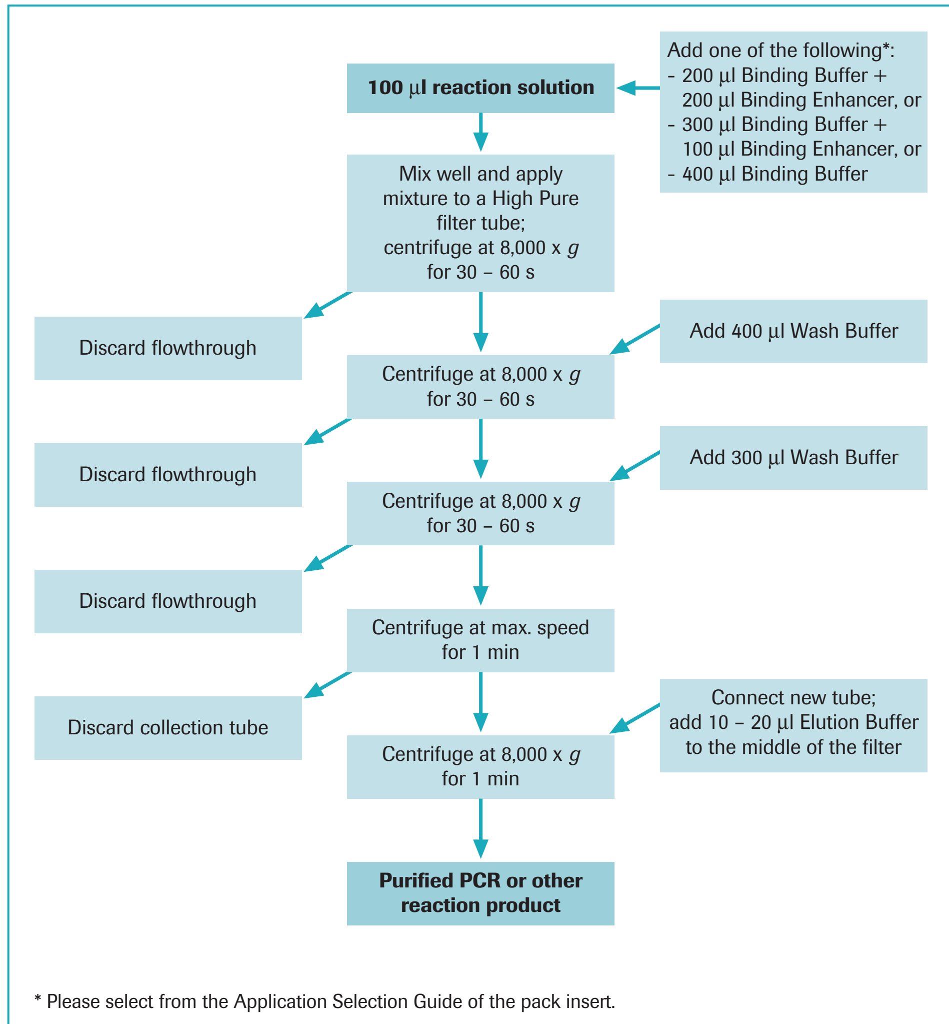
How to use the kit

I. Flow diagram



II. Kit contents

- Binding Buffer 20 ml or 80 ml
- Binding Enhancer 15 ml or 45 ml
- Wash Buffer 10 ml or 2 x 20 ml
- Elution Buffer 40 ml
- High Pure Micro Filter Tubes (50 or 200 tubes)
- Collection Tubes (50 or 200 tubes)

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III. Additional materials needed

The following additional reagents and equipment are required for cleaning up DNA fragments from solutions:

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 x *g* centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

The following additional reagents and equipment are required for cleaning up DNA fragments from agarose slices:

- Absolute ethanol
- Agarose
- TAE buffer (40 mM Tris-acetate, 1 mM EDTA) pH 8.4 or
- TBE buffer (89 mM Tris-borate, 2 mM EDTA) pH 8.4
- Electrophoresis equipment
- Sterile scalpel
- Standard tabletop microcentrifuge capable of 13,000 x *g* centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

IVa. Purification of PCR Products from Solution

Use the following procedure to remove low molecular weight DNA up to 70 bp from PCR or other reactions. For other applications please refer to Application Selection Guide in the pack insert

To process a larger sample (>100 µl), divide it into several 100 µl aliquots and process each as a separate sample.

Make sure that your sample is in a tube that can hold more than 500 µl. If the sample is > 100 µl, it should be in a 1.5 ml tube.

1. After the PCR is finished, adjust total volume of each sample to 100 µl by adding double dist. water.
   - To each 100 µl PCR sample, add:
   - 400 µl Binding Buffer
   - Mix sample well by vortexing (*e.g.*, vortex twice, for 4 s each).
   - Centrifuge the mixture briefly.
2. Insert one High Pure Filter Tube into one Collection Tube.
   - Using a pipette, transfer the sample from step 1 to the upper reservoir of the Filter Tube.
   - Centrifuge 30-60 s at 8,000 x *g* in a standard table top centrifuge at +15 to +25°C.
3. Disconnect the Filter Tube, and discard the flowthrough solution.
   - Reconnect the Filter Tube to the same Collection Tube.
4. Add 400 µl Wash Buffer to the upper reservoir.
   - Centrifuge 30-60 s at 8,000 x *g* (as above).

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5. Discard the flowthrough solution.
   - Reconnect the Filter Tube to the same Collection Tube.
   - Add 300 µl Wash Buffer.
   - Centrifuge 30-60 s at 8,000 x *g* (as above).

   This second (300 µl) wash step ensures optimal purity.
6. Discard the flowthrough solution.
   - Reconnect the Filter Tube to the same Collection Tube.
   - Centrifuge 1 min at maximum speed.
   - Discard the flowthrough solution and the Collection Tube.
   - Connect the Filter Tube to a clean 1.5 ml microcentrifuge tube.

   This step ensures complete removal of Wash Buffer.
7. Add 10 - 20 µl Elution Buffer to the center of the Filter Tube.
   - Centrifuge 1 min at 8,000 x *g*.

   Do not use water for elution, since alkaline pH is required for optimal yield.
8. The microcentrifuge tube now contains the purified DNA.

   If you plan to determine the A_{260} of the eluted DNA, first centrifuge the eluate for more than 1 min at maximum speed to remove residual glass fibers, which may interfere with the absorbance measurement. Use an aliquot of the supernatant to determine concentration

   Either use the eluted DNA directly or store the eluted DNA at +2 to +8°C or -15 to -25°C for later analysis.

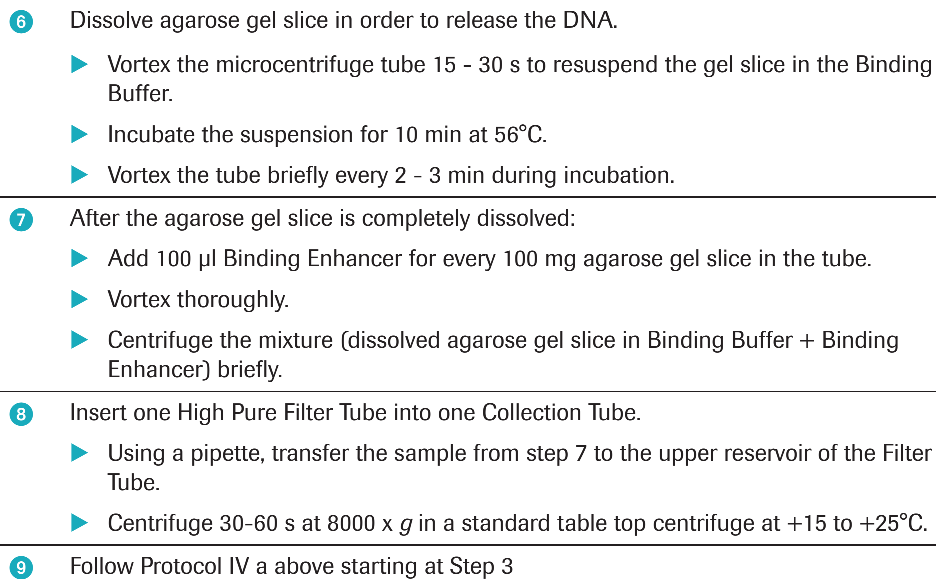
IVb. Purification of DNA Fragments from Agarose Gel

Use the following procedure to purify DNA from a 100 mg agarose gel slice.

1. Isolate DNA band of interest via agarose gel electrophoresis as follows:
   - Load PCR product mixture on a 0.8 - 2% agarose gel.
   - Use 1 x TAE or 1 x TBE as running buffer.
   - Electrophorese until DNA band of interest is separated from adjacent contaminating fragments.
2. Identify bands by staining gel with ethidium bromide or SYBR Green I Nucleic Acid Gel Stain.

   Wear gloves; ethidium bromide is a potent carcinogen.
3. Cut desired DNA band from gel using a scalpel or razor blade that has been sterilized with ethanol.

   Minimize volume of slice by placing gel on a UV light box (to make the DNA visible) and cutting the smallest possible gel slice that contains the desired DNA.
4. Preweigh an empty, sterile 1.5 ml microcentrifuge tube.
   - Place excised agarose gel slice in the sterile microcentrifuge tube.
   - Determine gel weight by reweighing the tube containing the gel slice and subtracting the weight of the empty tube.
5. To the microcentrifuge tube, add 300 µl Binding Buffer for every 100 mg agarose gel in the tube.



6. Dissolve agarose gel slice in order to release the DNA.
   - Vortex the microcentrifuge tube 15 - 30 s to resuspend the gel slice in the Binding Buffer.
   - Incubate the suspension for 10 min at 56°C.
   - Vortex the tube briefly every 2 - 3 min during incubation.
7. After the agarose gel slice is completely dissolved:
   - Add 100 µl Binding Enhancer for every 100 mg agarose gel slice in the tube.
   - Vortex thoroughly.
   - Centrifuge the mixture (dissolved agarose gel slice in Binding Buffer + Binding Enhancer) briefly.
8. Insert one High Pure Filter Tube into one Collection Tube.
   - Using a pipette, transfer the sample from step 7 to the upper reservoir of the Filter Tube.
   - Centrifuge 30-60 s at 8000 x *g* in a standard table top centrifuge at +15 to +25°C.
9. Follow Protocol IV a above starting at Step 3

IV. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 98 of this manual. For factors that may affect the High Pure PCR Cleanup Micro Kit, see page 100.

Typical results with the kit

Experiment 1

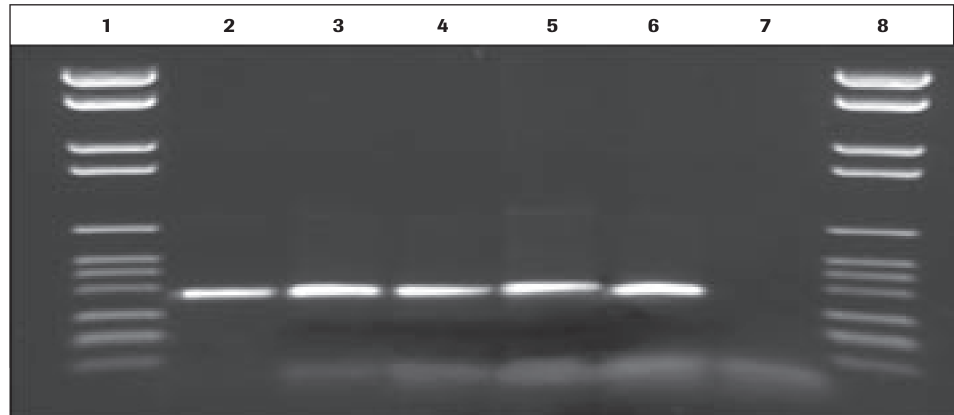


Figure 6: 1% agarose gel electrophoresis of 341 bp PCR product recovered in the presence of different amounts of Binding Enhancer.

Lane 1: Molecular weight marker VI
Lane 2: 0% Binding Enhancer
Lane 3: 10% Binding Enhancer
Lane 4: 20% Binding Enhancer
Lane 5: 40% Binding Enhancer
Lane 6: PCR without purification
Lane 7: PCR negative control (PCR without template)
Lane 8: Molecular weight marker VI

Experiment 2

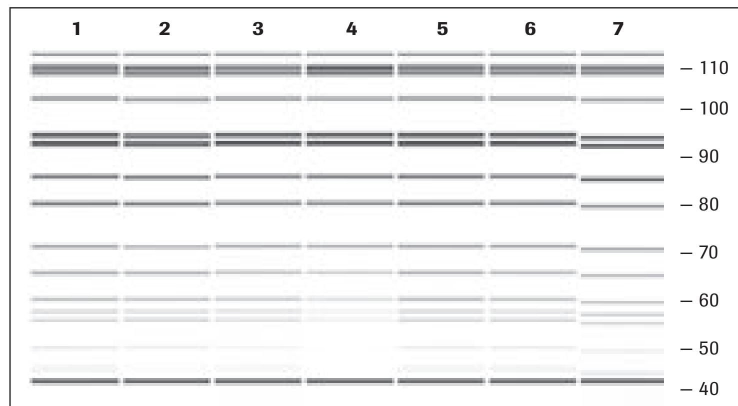


Figure 7: Electropherogram of the DNA fragment distribution obtained from a Agilent Bioanalyzer run (Agilent, USA).

Lane 1: Competitor Q
Lane 2: Competitor MN
Lane 3: Competitor P
Lane 4: Roche; 0% Binding Enhancer
Lane 5: Roche; 20 % Binding Enhancer
Lane 6: Roche: 40% Binding Enhancer
Lane 7: Molecular weight marker VIII, before purification

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References

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