

High Pure 96 UF Cleanup Kit

for high-throughput purification of PCR products by ultrafiltration

Cat. No. 04 422 694 001 (2 x 96 purifications)

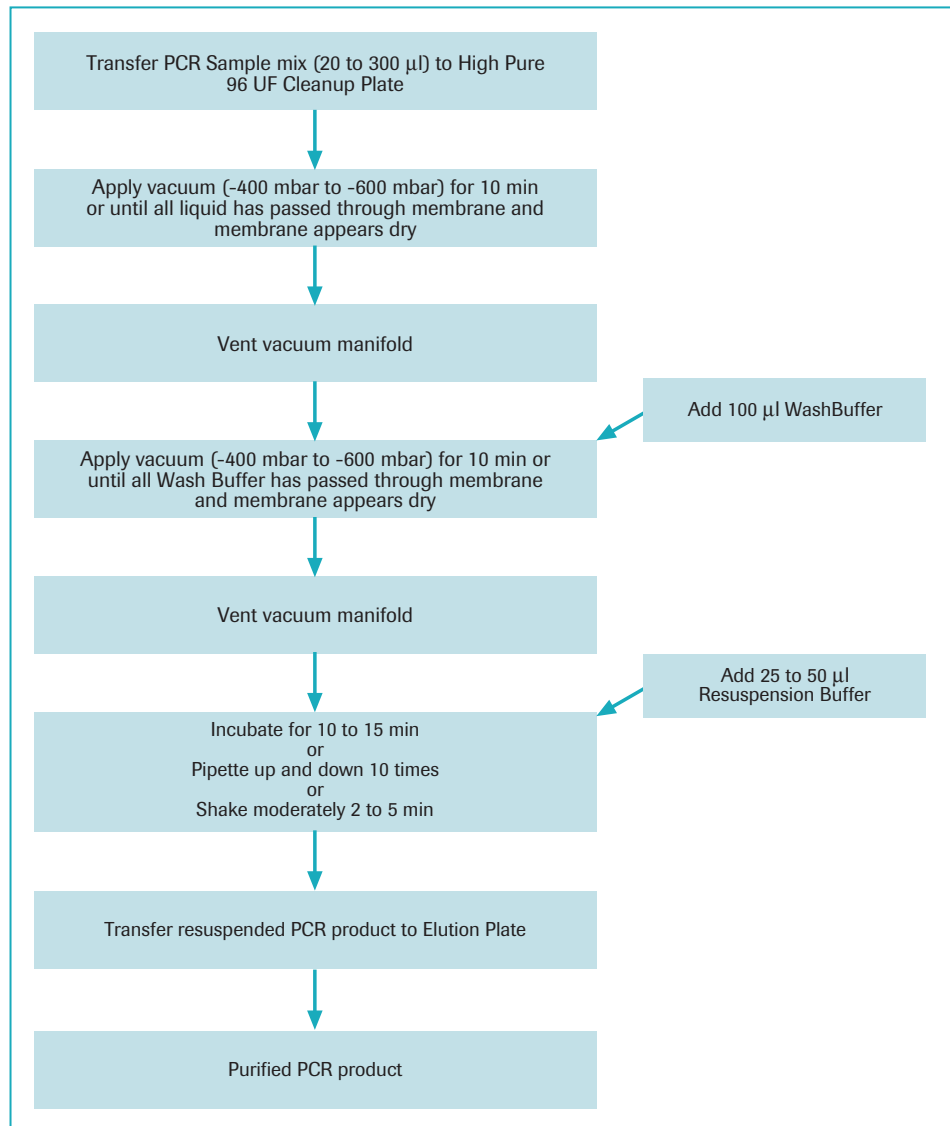
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Principle	The kit uses ultrafiltration and size exclusion to separate small from large molecules. Small contaminants (dNTP's, primer, primer dimers, salts, etc.) that may interfere with downstream applications readily pass through a membrane (and into a waste container), while PCR products remain atop the membrane. The purified DNA can either be washed to ensure complete removal of contaminants or resuspended in buffer for immediate use in downstream procedures.
Starting material	The kit can recover 20 - 300 µl PCR products that range from 100 bp to >10 kb.
Application	Use the High Pure 96 UF Cleanup Kit to quickly remove dNTPs, salts, primers and other small molecules from your PCR products, making them suitable for: microarray spotting, labeling, restriction digest, automated fluorescent sequencing, and cloning.
Time required	<ul style="list-style-type: none"> Manual processing on vacuum manifolds or microplate centrifuges takes approx. 20 min for 96 samples. Automated processing depends on the liquid handling instrument used; it can be as little as 15 min.
Results	<ul style="list-style-type: none"> The purity of the recovered DNA is shown by its A_{260}/A_{280} ratio, which is 1.8 ± 0. The amount of DNA recovered depends on the elution volume and the length of the PCR product. Typically, 90% of a 1 kb fragment can be recovered in 25 µl elution buffer. The recovery rate depends on the length of the amplification product: ≥ 150 bp recovery $\geq 40\%$; 1500 bp to 4500 bp recovery $\geq 90\%$; and 8000 bp recovery $\geq 80\%$.
Benefits	<ul style="list-style-type: none"> Flexible; works well with vacuum manifolds, microplate centrifuges, and common liquid handling instruments. Simple; provides all necessary components in ready-to-use form. Suitable for high volume laboratories; purifies up to 96 samples in less than 20 minutes. Avoids cross-contamination; isolates samples in 96 individual wells to ensure that no well-to-well or aerosol „cross-talk“ occurs. Convenient; samples are applied and removed from the top. Efficient; gives excellent recovery – even with small (100 bp) PCR fragments.

How to use the kit

I. Flow diagram

Using Vacuum Manifold



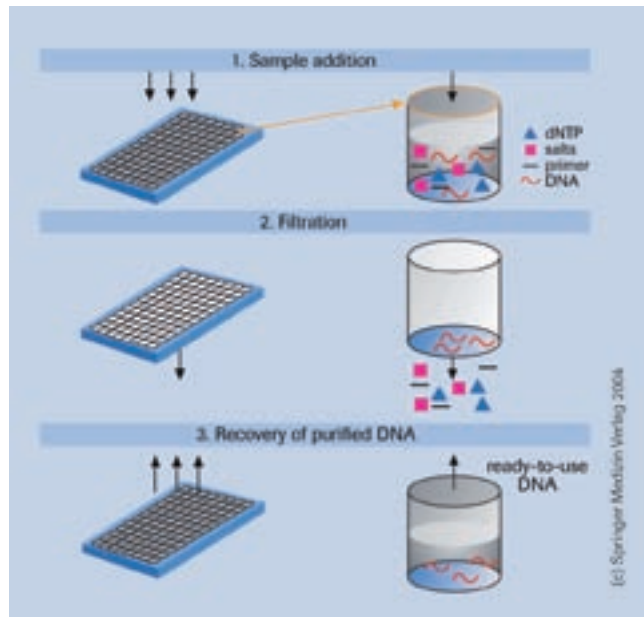
II. Kit content

- 2 High Pure 96 UF Cleanup Plates
- 100 ml Wash Buffer
- 2 Waste Plates
- 100 ml Resuspension Buffer
- 2 Elution Plates with self-adhering foil

III. Additional materials needed

- Vacuum manifold OR
- Microtiter plate centrifuge capable of 4500 x g centrifugal force (e.g., Beckman Coulter Allegra 25R)
- Multichannel pipette (optional)

Schematic overview of PCR product purification



IV. Isolation Protocol

IVa. Isolation Protocol Using a Vacuum Manifold


The protocol is designed for reaction volumes of 20 to 100 μl . You will have to increase the filtration time to process larger volumes (up to 300 μl). This manual isolation protocol may be automated for use with common liquid handling systems.

- 1 Pipette 20 to 100 μl PCR reaction mix directly onto the membrane in one well of the High Pure 96 Cleanup Plate.
 - ⚠ *Avoid dispensing sample onto the inner wall of the well. Unused wells of the plate may be left open. They do not need to be sealed.*
- 2 Remove contaminants by ultrafiltration. Place plate on a suitable vacuum manifold (such as those listed in the package insert) and apply vacuum until all liquid has passed through the membrane.
 - ⚠ *Indicated times are approximate. Depending on the sample, the time required may increase or decrease. Make sure that all liquid has passed through the membrane (membrane will appear dry). After all liquid has been filtered, dry the membrane for an additional 30–60 s by continuing to apply vacuum. 400 to 600 mbar for 10 to 15 min.*



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- 3 Optional wash step: Vent the vacuum manifold 60 to 90 s. Dispense 100 μ l Wash Buffer into each well. Apply vacuum 400 to 600 mbar for 10 to 15 min to draw liquid through the membrane until all Wash Buffer has passed through. Apply vacuum for an additional 30–60 s.

 Typically, you do not need to wash the collected DNA. However, you have problems with downstream applications or want to ensure removal of small molecules, we recommend using the extra wash step.

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- 4 Vent the vacuum manifold 60 to 90 s. Add 25 to 100 μ l Resuspension Buffer directly to the center of the membrane in each well.

 The dead volume of the membrane is 3–4 μ l


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- 5 Incubate the Resuspension Buffer on the membrane for 5–10 min OR resuspend DNA by pipetting the liquid up and down 5–10 times mixing OR resuspend DNA by placing the Cleanup Plate on a microplate shaker for 2 to 5 min. (We recommend using 50 μ l Resuspension Buffer for resuspension on a shaker).

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- 6 Use a pipette to remove the resuspended, purified product from Cleanup Plate and transfer it to a clean Elution Plate.


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- 7 Use purified product directly in downstream applications or seal Elution Plate with foil for storage of the resuspended DNA samples.
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Vib. Isolation Protocol Using a Microtiter Plate Centrifuge


- 1 Pipette 20 to 100 μ l PCR reaction mix directly onto the membrane in one well of the High Pure 96 UF Cleanup Plate.

 Avoid dispensing sample onto the inner wall of the well. Unused wells of the plate may be left open. They do not need to be sealed.

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- 2 Place the plate onto the Waste Collection Plate (supplied with the kit). Place the plate „sandwich“ in a suitable centrifuge (such as those listed in package insert) and spin at 4,500 x *g* for 5–10 min at 15–25°C.

 Indicated times are approximate. Depending on the sample, the time required may increase or decrease. Make sure that all liquid has passed through the membrane (membrane will appear dry).

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- 3 Wash the membrane by adding 100 μ l Wash Buffer directly to the center of the membrane in each well (without separating the Cleanup Plate/Waste Plate sandwich). Centrifuge the assembly at 4,500 x *g* for 5–10 min at 15–25°C.

 We strongly recommend performing the wash step when you are using centrifugation to purify the DNA. After the first spin, minute amounts of liquid (containing salts etc.) will remain on top of the membrane and contaminate the resuspended sample.

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- 4 Add 25 to 100 μ l Resuspension Buffer directly to the center of the membrane in each well.

 The dead volume of the membrane is 3–4 μ l.

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- 5 Incubate the Resuspension Buffer on the membrane for 5–10 min OR Resuspend DNA by pipetting the liquid up and down 5–10 times mixing OR Resuspend DNA by placing the Cleanup Plate on a microplate shaker for 2 to 5 min. (We recommend using 50 μ l Resuspension Buffer for resuspension on a shaker).

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- 6 Use a pipette to remove the resuspended, purified product from the Cleanup Plate and transfer it to the clean Elution Plate.

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- 7 Use purified product directly in downstream applications or seal Elution Plate with foil for storage of the resuspended DNA samples.
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V. Factors that may affect the High Pure 96 UF Cleanup Kit

If you get...	Then, the cause may be...	And you should...
Low DNA recovery	Elution conditions not optimal	<ul style="list-style-type: none"> ▶ Increase number of mixing steps. ▶ Increase incubation time.
	Did not use enough Resuspension Buffer	▶ Recommended amount of Resuspension Buffer: at least 25 µl for manual purification or at least 50 µl for automated purification or resuspension on a shaker
	DNA fragments dried on membrane	▶ Dispense Resuspension Buffer or nuclease-free water onto membrane and incubate 15 to 30 min at 15 to 25°C to allow DNA to rehydrate before trying to recover it.
	Small PCR Product	<ul style="list-style-type: none"> ▶ Increase number of mixing/resuspension steps. ▶ Pipette the resuspended DNA up and down at least 5-10 times.
Contaminated samples	Samples not filtered completely	▶ Force samples completely through filter until membrane appears dry and shiny
	Sample remains on the inner wall of well	<ul style="list-style-type: none"> ▶ Dispense samples directly onto the membrane. Make sure that no sample material sticks to the side of the wells. ▶ Avoid letting the pipette tip touch the wall during automated purification. ▶ Perform optional washing step
	PCR buffer contains detergents	<ul style="list-style-type: none"> ▶ Although detergents are effectively removed by ultrafiltration on the High Pure 96 UF Cleanup Plate, the presence of detergent in commercially available PCR buffers may lead to increased filtration time. ▶ Use of detergent-free PCR buffers is recommended.

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Typical results with the kit

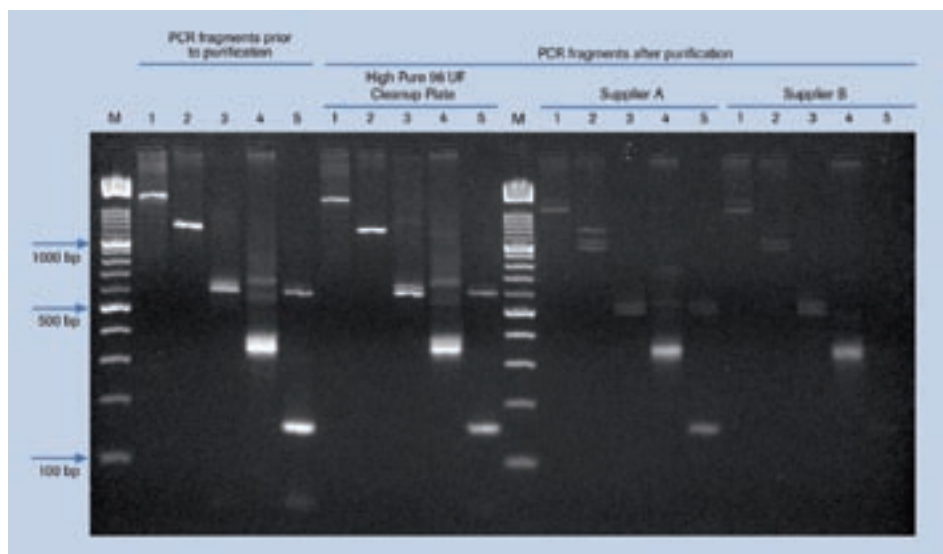


Figure 33: Purification of small and large PCR products with the High Pure 96 UF Cleanup Kit and competing products. Equal amounts of sample were analyzed on a gel after they were purified with the indicated products. Lanes 1: 1.7 kb PCR fragment, lanes 2: 1.2 kb PCR fragment, lanes 3: 600 bp PCR fragment, lanes 4: 350 bp PCR fragment, lanes 5: 165 bp PCR fragment. (M: DNA Molecular Weight marker XIV.)

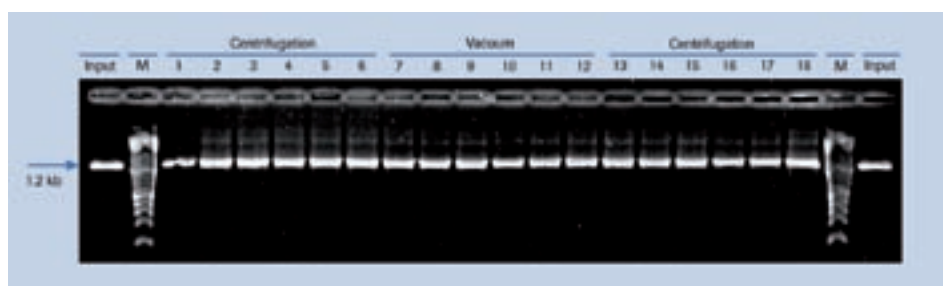


Figure 34: Comparison of purification via vacuum (lanes 7-12) and centrifugation (lanes 1-6, 13-18). Equal amounts of sample were analyzed on a gel after they were purified with the High Pure UF 96 Cleanup Kit using the indicated method. Samples analyzed in lanes 1-6 and 13-18 were purified on different High Pure 96 UF Cleanup Plates. Lane marked „Input“ contained a sample of the PCR product obtained before purification. (M: DNA Molecular Weight Marker XIV.)

Reference

Victor M and Walter T. (2004), *Biochemica* **3**; 13 – 15