

High Pure 96 UF Cleanup System – Ultrafiltration for Fast and Efficient Recovery of PCR Fragments

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Purification of PCR fragments is a prerequisite for the success of demanding downstream applications such as fluorescent sequencing, single nucleotide polymorphism (SNP) genotyping, and microarray spotting. Besides high purity, high sample throughput is becoming increasingly important. The new High Pure 96 UF Cleanup System is based on ultrafiltration technology in a 96-well format and yields pure DNA in mid to high throughput. The High Pure 96 UF Cleanup System is available in two formats:

- ➔ A convenient kit including all material needed for processing the High Pure Cleanup Plate
- ➔ A Cleanup Plate-only format for the experienced and/or high-throughput user

The Working Principle: Size Exclusion

The High Pure 96 UF Cleanup System works on the principle of size exclusion to separate the desired DNA fragments from salts and other contaminants (Figure 1). A sturdy ultrafiltration membrane – acting as a molecular sieve – is inserted at the bottom of each well of a 96-well plate in standard microtiter plate format. After the PCR mix has been applied to each well, liquid, as well as salts, dNTPs, and primers are filtered to waste by the application of either vacuum or centrifugation. The DNA remains on top of the membrane and can either be further purified by an optional washing step or directly recovered using resuspension buffer. Due to the small dead volume (only 3–4 μ l) and the unique design of the membrane, DNA recovery is highly efficient even for small fragments.

Since the hydrophilicity of the membrane is achieved without use of detergents, none are present in the recovered nucleic acid solution and the nucleic acids can be used directly, e.g., in microarray spotting.

The procedure is a simple one-step protocol that eliminates binding, washing, and elution steps and the need for using alcohol or other hazardous substances. Figure 1 gives a schematic overview of the procedure and principle underlying PCR fragment purification using the High Pure 96 UF Cleanup System.

Use with Vacuum or Centrifuge

Owing to its one-piece design and standardized size, the High Pure 96 Ultrafiltration Plate can be processed on most of the commonly used vacuum manifolds. Similarly, most standard tabletop centrifuges equipped with a microplate holder can also be used to process the High Pure 96 UF Cleanup System (centrifuges should be capable of generating a relative centrifugal force [RCF] of 4,500 $\times g$). As a result of the easy accessibility of the wells from the top for loading as well as for recovery of the purified DNA, PCR fragment purification can be easily automated on standard liquid-handling machines.

Efficient Purification of PCR Fragments of Different Sizes

DNA fragments of different lengths were generated using the Pwo Master. Equal aliquots of the reaction were diluted to a final volume of 100 μ l (recommended volume for an even filtration) and applied to the wells of a High Pure 96 UF Cleanup Plate. Then, the Cleanup Plate was

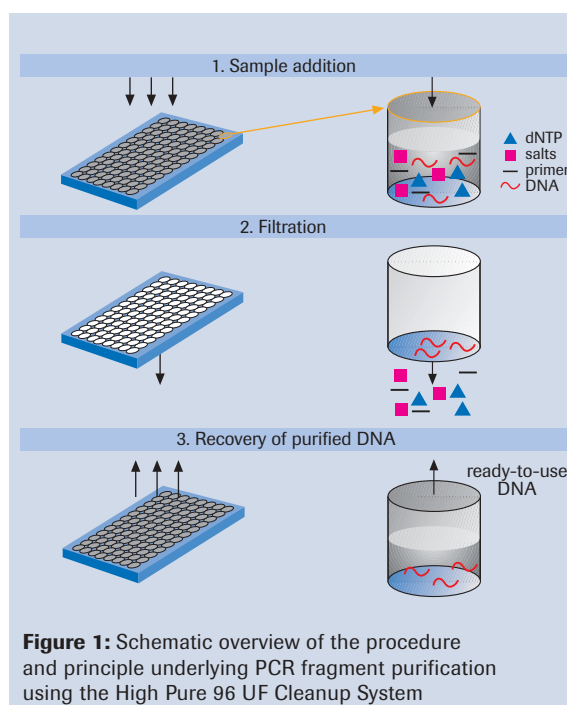


Figure 1: Schematic overview of the procedure and principle underlying PCR fragment purification using the High Pure 96 UF Cleanup System

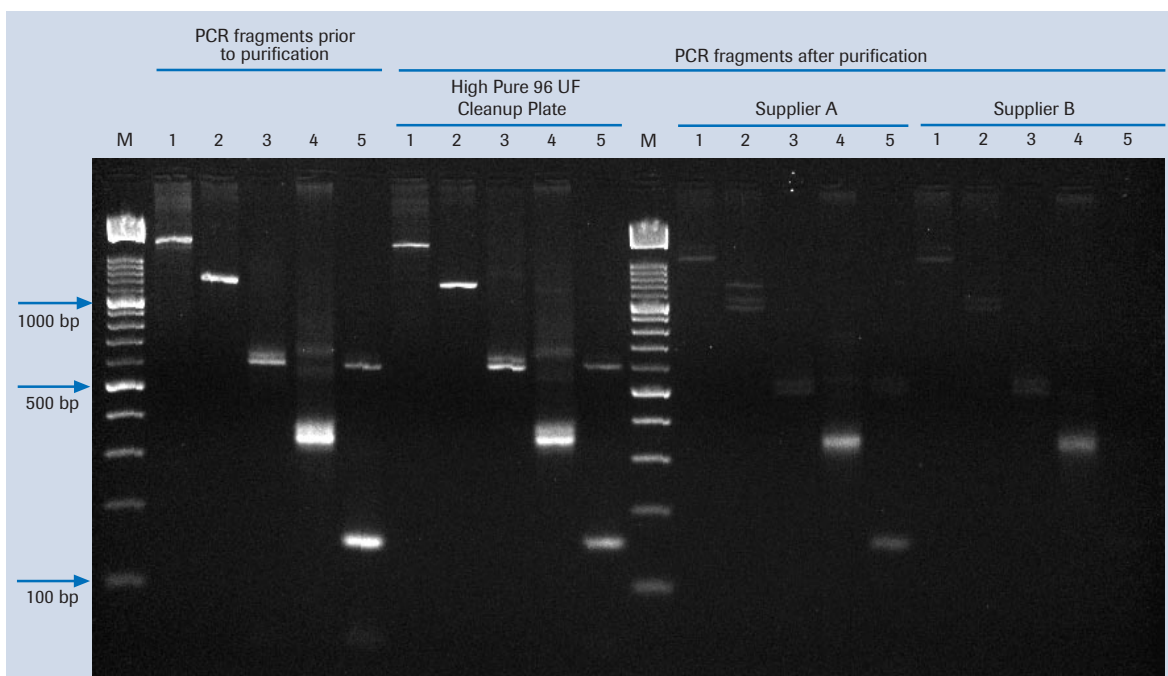


Figure 2: Equal amounts of sample were loaded per well after purification using the indicated products. Lanes 1: 1.7-kb PCR fragment, lanes 2: 1.2-kb PCR fragment, lanes 3: 600-bp PCR fragment, lanes 4: 350-bp PCR fragment, lanes 5: 165-bp PCR fragment (M, DNA Molecular Weight Marker XIV).

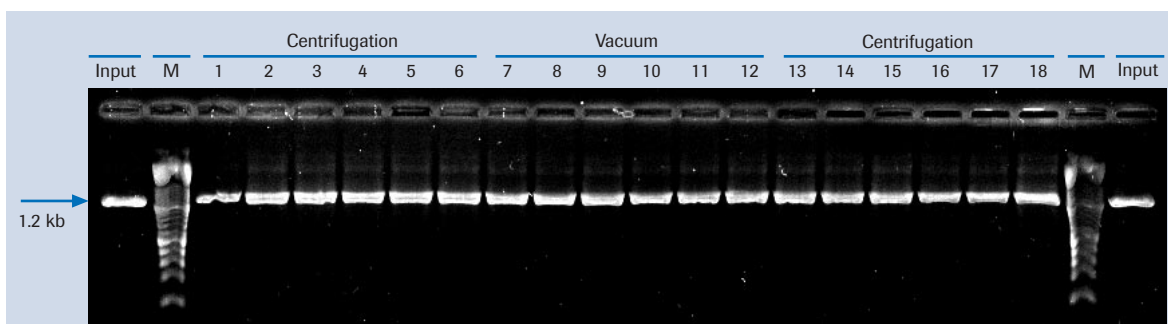


Figure 3: Equal amounts of sample were loaded per well after purification by either vacuum (lanes 7-12) or centrifugation (lanes 1-6, 13-18). Samples analyzed in lanes 1-6 and 13-18 were purified on different High Pure 96 UF Cleanup Plates. PCR product prior to purification is indicated by Input (M, DNA Molecular Weight Marker XIV).

placed on top of a suitable vacuum manifold. Vacuum was applied for approximately 10 minutes (~400 mbar), resulting in the complete removal of contaminating salts, dNTPs, primers, and primer-dimers. Unused wells do not have to be sealed; this allows for partial use of the 96-well Cleanup Plate. DNA trapped on the surface of the membrane was washed with 100 μ l wash buffer by vacuum application for approximately 10 minutes (optional step). Then 50 μ l resuspension buffer was added to the DNA. After a 5-minute incubation, PCR fragments were recovered by pipetting up and down ten times using a multichannel pipette.

To evaluate the performance of the new kit, two products from different suppliers were also used for purification of DNA fragments according to the manufacturer's instructions.

Equal amounts of the reaction mix prior to and after purification were analyzed by agarose gel electrophoresis. As shown in Figure 2, application of the High Pure 96 UF Cleanup System for purification of PCR fragments results in highly efficient recovery of the applied DNA, covering a broad range of sizes, down to 165 bp. Note that contaminating primer-dimers are also efficiently removed.

Robust Recovery Using Either Centrifugation or Vacuum

Aliquots containing equal amounts of a 1.2-kb PCR fragment were processed with the High Pure 96 UF Cleanup System using a vacuum manifold as described above. Additional aliquots (100 μ l) were purified using a Heraeus

Table 1: **Characteristics of the High Pure 96 UF Cleanup System**

Specification	Value	Comment
Sample volume	20-300 µl/well	100 µl recommended sample volume, filtration times increase as a function of higher volumes
Recovery volume	≥ 25 µl	50 µl recommended for automated use
Purity	OD _{260nm} /OD _{280nm}	1.7-1.9
Dead volume	3-4 µl	
Filtration time	10 minutes for 100-µl sample	Values apply to a vacuum of -400 to -600 mbar and may increase for higher volumes
Centrifugation time	10 minutes for 100-µl sample	Values apply to 4,500 x <i>g</i> relative centrifugal force, times may increase with reduced centrifugal force or increased volume
DNA recovery	≥ 150 bp ≥ 40% 1,500 bp ≥ 90% 8,000 bp ≥ 80%	as determined by densitometric scanning after gel electrophoresis

Multifuge 3L-R. Samples were applied to the wells of a High Pure 96 UF Cleanup Plate. Then the Cleanup Plate was placed on top of a Waste Plate (deep-well plate in microtiter plate format included in the kit). The resulting plate sandwich was centrifuged for 10 minutes (4,500 x *g*). The DNA was washed with 100 µl wash buffer by another round of centrifugation (10 minutes, 4,500 x *g*); this washing step is recommended when using a centrifuge. PCR fragments were recovered by the addition of 50 µl resuspension buffer followed by a 5-minute incubation at room temperature and repeated pipetting up and down using a multichannel pipette.

Equal volumes of solution containing the purified fragments were subjected to agarose gel electrophoresis. Figure 3 shows that the High Pure 96 UF Cleanup System results in highly efficient and robust recovery of PCR fragments using either vacuum or centrifugation as a means of purification.

Conclusion

The High Pure 96 UF Cleanup System is a convenient solution for efficient and robust purification of PCR fragments in a high-throughput mode (Table 1). In only

20 minutes and little hands-on time, 96 samples can be processed simultaneously using either a vacuum manifold, a centrifuge, or an automated system. Due to the unique properties of the Cleanup Plate, the purified DNA can be directly used in downstream applications such as e.g. fluorescent sequencing, SNP analysis, and microarray spotting. ■

Product	Pack Size	Cat. No.
High Pure 96 UF Cleanup Kit	2x 96 reactions	04 422 694 001
High Pure 96 UF Cleanup Plates	10x 96-well plates	04 422 716 001
Agarose MS	100 g	11 816 586 001
	500 g	11 816 594 001
Agarose MP	100 g	11 388 983 001
	500 g	11 388 991 001
DNA Molecular Weight Marker XIV (100 bp ladder)	50 µg (1 A ₂₆₀ unit)	11 721 933 001

