PUREBIOTECH LLC
752A Lincoln Blvd.
Middlesex, NJ 08846

Toll Free: 866-252-7771
Phone: 732-469-7771 Fax: 732-469-7782
Web: www.purebiotechllc.com

96-Well PCR Purification Kit
Product Information

Product:	96-Well PCR Purification Kit
Protocol No:	61.002
Storage & Stability:	Stable for 1 year at Room Temperature

PRODUCT NO.	DESCRIPTION	UNIT
PCR-25-01	96-Well PCR Purification Kit	Starter Kit (1 x 96)
PCR-25-10		Mini Kit (10 x 96)
PCR-25-50		Midi Kit (50 x 96)
PCR-25-100		Maxi Kit (100 x 96)

Introduction

Congratulations on taking a big step towards improving the economies of scale, quality and speed of your genomics research. This booklet contains a complete set of protocols outlining the steps, principles, materials and equipment needed to use this kit. The PCR Purification Kit provides purified PCR products of the finest quality and purity for a broad spectrum of downstream applications in genomics at a fraction of the cost of leading vendors.

Product Performance

The PCR Purification Kit combines the ease and convenience of advanced separation technology chemistry with the speed of a multiplexed 96-well microplate centrifugation format. In the presence of Binding Buffer, double-stranded DNA selectively binds to the Super Filter membrane. The unique chemical composition of the Binding Buffer provides a pale blue appearance that helps facilitate sample mixing and tracking, and assists in the monitoring of the subsequent wash steps. The Wash Buffer selectively rinses away contaminants such as primers, nucleotides, salts, proteins and other impurities. In the final step, a mild buffer containing 0.1 mM EDTA is used to elute the bound PCR product, yielding a highly purified DNA preparation.

Advantages of the advanced system include:

- No ethanol precipitations
- No organic solvents
- Reduced plastic consumption
- Low cost ($0.42 per sample for the Maxi Kit)
- Environmentally friendly buffers
- Mild vacuum and centrifugation requirements
- Easy sample tracking

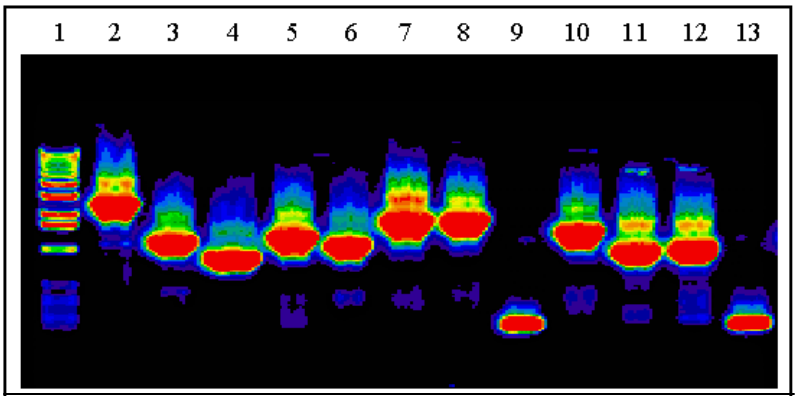

Figure 1: PCR products purified with our kit. Human cDNA inserts were amplified by PCR, purified with our 96-Well PCR Purification Kit and 10% of each sample (lanes 2 – 13), together with molecular weight markers (lane 1), were fractionated on 0.8% agarose, stained with ethidium bromide and photographed. Fluorescent image is represented in grey scale.

The 96-Well PCR Purification Kit was designed by experts specifically for the genomics, biotechnology and pharmaceutical research communities. The yield and purity of the resulting PCR products, coupled with low level of cross-contamination and high-throughput, define a kit that routinely provides:

>90% yield of amplified product
>99.9% purity
>1,000 purified PCR products per day
<0.01% well-to-well contamination

Because of the high quality of performance, DNA purified with the kit is suitable for a wide range of downstream applications that require a large number of highly purified PCR products. These applications include:

- DNA Microarrays
- DNA Chips
- Gene Arrays
- High Density Filters
- Ink-Jetting Delivery
- Gene Mapping
- DNA Sequencing
- Deletion Studies
- Other Genomics Applications

KIT CONTENTS

Starter Kit (1 x 96)
96-Well Super Filter (1)
Labeled Microplate and Cover (1)
Unlabeled Microplate (1)
Binding Buffer (15 ml)
Wash Buffer (150 ml)

Mini Kit (10 x 96)
96-Well Super Filter (10)
Labeled Microplate and Cover (10)
Unlabeled Microplate (10)
Binding Buffer (150 ml)
Washing Buffer (3 x 500 ml)

Midi Kit (50 x 96)
96-Well Super Filter (50)
Labeled Microplate and Cover (50)
Unlabeled Microplate (50)
Binding Buffer (2 x 280 ml)
Wash Buffer (5 x 1,000 ml)

Maxi Kit (100 x 96)
96-Well Super Filter (100)
Labeled Microplate and Cover (100)
Unlabeled Microplate (100)
Binding Buffer (4 x 280 ml)
Washing Buffer (10 x 1,000 ml)

Equipment Requirements

- Standard 96-well vacuum filter block
- Multi-channel pipetting device (capable of 25-150 µl)
- Multi-channel pipetting device (capable of 350 µl)
- Microplate centrifuge with vacuum capabilities

Short Protocol (Steps 1-15)

1. Obtain a 96-well plate of PCR products concentrated to 25 µl per well.
2. Add 100 µl per well of Binding Buffer.
3. Mix the PCR sample and Binding Buffer by pipetting 10 times.
4. Transfer the 125 µl contents of each well to the Super Filter positioned on a 96-well vacuum block.
5. Apply a gentle vacuum to allow binding of the DNA to the Super Filter membrane (impurities flow through).
6. Wash each well immediately with three (3) successive 350 µl volumes of Wash Buffer under a gentle vacuum.
7. Apply a full vacuum for 3 minutes to dry the membrane.
8. Remove the Super Filter from the vacuum block and place it on an unmarked 96-well microplate.

9. Centrifuge the two plates for 5 minutes at ambient temperature in a microplate centrifuge (~500 x g) to remove residual Wash Buffer.

10. Discard the unmarked microplate and its contents.

11. Transfer the Super Filter 100 onto a marked 96-well microplate.

12. Add 100 µl per well of 0.1X TE (pH = 8.0).

13. Wait 1 minute to allow re-wetting of the membrane.

14. Centrifuge for 5 minutes at ambient temperature in a microplate centrifuge (~500 x g) to elute the purified PCR product.

15. Evaporate the ~ 80 µl DNA samples to dryness by vacuum centrifugation and re-suspend the DNA (>5 µg) in buffer of choice.

Complete Protocol (1-14)

1. Amplify double-stranded DNA products by use of the polymerase chain reaction (PCR) under the conditions of choice. Use 25-100 µl reaction volumes in a 96-well format. Following PCR amplification, reduce the volume to 25 µl per well by use of a 96-well microplate centrifuge equipped with vacuum capabilities. Approximately 30 minutes with heating is required to reduce a plate of 100 µl reactions to 25 µl per well. The volume reduction step reduces buffer volumes and thus greatly minimizes well-to-well contamination that may occur during sample mixing and transfer. If samples are accidentally taken to dryness, add 25 µl per well of H_2O and re-suspend by pipetting up and down with a multi-channel pipetting device.

2. Add 100 µl of Binding Buffer to each 25 µl PCR sample with a multi-channel pipetting device fitted with 200 µl tips. The Binding Buffer should be added gently to avoid splashing the contents from well to well.

3. Mix the PCR reaction with the Binding Buffer by pipetting the mixture up and down 10 times with a multi-channel pipetting device set at 100 µl. Plugged pipette tips can be used to avoid contamination by aerosols generated during mixing. The pale blue dye in the Binding Buffer can be used as a visual aid to monitor mixing.

4. Transfer the 125 µl contents of each well to the Super Filter positioned on a 96-well vacuum block. This transfer step is most efficiently made with a multi-channel pipetting device set to 150 µl. A pipette setting of 150 µl (instead of 125 µl) ensures complete transfer of the entire contents of each well. To maximize recovery, contents should be pipetted directly onto the surface of the Super Filter membrane.

5. Apply a gentle vacuum to allow binding of the PCR product to the Super Filter membrane. Primers, nucleotides, single-stranded products, salts, dyes and other impurities pass through the Super Filter into the waste reservoir at the bottom of the vacuum filtration block. Following the filtration step, the Super Filter membrane filter should take on a pale blue appearance. Proceed immediately to the wash step. Excessive drying of the Super Filter at this point can result in the binding of impurities to the membrane.

6. Wash the Super Filter with three (3) successive 350 µl aliquots of the Wash Buffer under a gentle vacuum to remove remaining contaminants and impurities trapped in the filter membrane. The 3 washes are best carried out with a multi-channel electronic pipetting device capable of delivering a 1,050 µl total volume (3 x 350 µl). Care should be taken not to splash Wash Buffer from well to well. During the wash steps, contaminants and impurities flow through the Super Filter and collect in the waste reservoir at the bottom of the filter block. The DNA remains bound to the Super Filter membrane. The pale blue dye should elute with the other contaminants during the wash steps. Following the final wash, the Super Filter membrane should regain its original white appearance.

7. Apply a full house vacuum for 3 minutes to dry the Super Filter membrane thoroughly. The PCR product remains attached to the Super Filter at this point. The membrane drying step serves to remove trace amounts of Binding Buffer, thereby increasing the purity and yield of the DNA product.

8. Remove the Super Filter from the vacuum filter block and place it on an unmarked 96-well microplate. Be sure to position the Super Filter accurately over the corresponding wells on the microplate. Accurate positioning minimizes the chance of contamination between adjacent wells that might arise from sliding the Super Filter across the microplate.

9. Transfer the two plates to a microplate centrifuge and spin for 5 minutes at ~ 500 x g. During centrifugation, residual Wash Buffer is transferred from the Super Filter into the unmarked 96-well plate. This results in the complete drying of the Super Filter membrane, increasing the yield and purity of the DNA. All centrifugation steps should be carried out at ambient temperature (25°C). Performing the centrifugation steps at elevated temperatures (>37°C) can cause irreversible binding of the DNA to the Super Filter membrane, resulting in reduced yield.

10. Following the 5 minute centrifugation step, remove the two microplates from the centrifuge and discard the unmarked microplate (bottom plate) and its contents. At this point, the Super Filter 96-well plate contains the PCR product bound to the membrane.

11. Place the Super Filter onto a marked 96-well microplate in the correct orientation. The numbers and letters on the Super Filter should correspond to those on the marked microplate. Be sure the plates are oriented correctly! The incorrect placement of the Super Filter on the microtiter plate will lead to catastrophic errors in sample tracking.

12. Re-hydrate the Super Filter by adding 100 µl per well of 0.1X TE (pH = 8.0) with a multi-channel pipetting device. For maximal DNA recovery, be sure to add the 0.1X TE directly onto the surface of the Super Filter membrane. The mild elution buffer (1 mM Tris-Cl, 0.1 mM EDTA) is used to minimize interference of the buffer in downstream applications. Let the Super Filter membrane sit for 1 minute at ambient temperature. Incubation periods in excess of 1 minute do not increase DNA yield. Shorter incubations can reduce yield.

13. Transfer the two microplates to a microplate centrifuge and spin for 5 minutes at ~ 500 x g. Centrifugation results in the elution of the PCR product from the Super Filter membrane and complete transfer of the contents into the marked 96-well microtiter plate (bottom plate). This step should be carried out at ambient temperature (25°C). Performing this step at elevated temperatures can reduce yield.

14. Remove the two plates from the microplate centrifuge and discard the Super Filter. The marked microplate (bottom plate) should contain highly purified PCR product at a concentration of 60-90 µg per ml in ~ 80µl
0.1X TE. The yield should be >90%. Evaporate the samples to dryness by vacuum centrifugation, re-suspend in buffer of choice and seal the samples for future use with microplate cover.

Troubleshooting Tips

The PCR Purification Kit is designed for high-throughput isolation of double-stranded PCR products ranging in size from 0.1-5.0 kb. Impurities such as nucleotides, primers, salts, dyes and other contaminants do not bind to the Super Filter membrane.

Reduced Yield:

- Inefficient PCR Amplification.
- Binding Buffer not added to PCR reaction products prior to filtration to filtration through the Super Filter.
- Incomplete drying of the Super Filter following the three wash steps.
- Salt-containing buffer instead of 0.1X TE (pH 8.0) used to elute the PCR products.

Contaminants in Purified DNA Sample:

- Super Filter subjected to elevated temperatures while containing bound PCR product.
- Incomplete washing of Super Filter.
- Impurities present in 0.1X TE (pH 8.0) elution buffer.

For research use only.

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or e-mail us at info@purebiotechllc.com.