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

384-Well PCR Purification Kit
Product Information

Product:	384-Well PCR Purification Kit
Protocol No:	59.002
Storage & Stability:	Stable for 1 year at Room Temperature

PRODUCT NO.	DESCRIPTION	UNIT
PCR-384-01	384-Well PCR Purification Kit	Starter Kit (1 x 384)
PCR-384-10		Mini Kit (10 x 384)
PCR-384-50		Midi Kit (50 x 384)
PCR-384-100		Maxi Kit (100 x 384)

Introduction

Congratulations on taking a big step towards improving the economies of scale, quality and speed of your genomics research. Attached is a complete set of protocols outlining the steps, principles, materials and equipment needed to use the 384-Well PCR Purification Kit. The 384-Well PCR Purification Kit provides purified PCR products of the finest quality and purity for a broad spectrum of downstream applications in geno mics at a fraction of the cost of leading vendors.

Product Performance

The 384-Well PCR Purification Kit combines the ease and convenience of advanced separation technology chemistry with the speed of a multiplexed 384-well microplate centrifugation format. In the presence of Binding Buffer, double-stranded DNA selectively binds to the 384 Well 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 EDTA is used to elute the bound PCR product, yielding a highly purified DNA preparation.

Advantages of this product include:

- 384 samples at a time
- Low cost
- Environmentally friendly buffers
- No organic solvents
- Mild vacuum and centrifugation requirements
- Reduced plastic consumption
- Easy sample tracking
- No ethanol precipitations

The 384-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
>3,840 purified PCR products per day
<0.01% well-to-well contamination

Because of the high quality of performance, DNA purified with the 384-Well PCR Purification Kit is suitable for a wide range of downstream applications that require a large number of highly purified PCR products. These applications include:

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

Kit Contents:

Starter Kit (1 x 384):
384-Well Super Filter (1)
Binding Buffer (75 ml)
Microplate 384 well with cover (1)
Microplate 384 well w/o cover (1)
Wash Buffer (150 ml)

Mini Kit (10 x 384):
384-Well Super Filter (10)
Microplate 384 well with cover (10)
Microplate 384 well w/o cover (10)
Binding Buffer (750 ml)
Wash Buffer (1,500 ml)

Midi Kit (50 x 384):
384-Well Super Filter (50)
Microplate 384 well with cover (50)
Microplate 384 well (50)
Binding Buffer (3.5 liters)
Wash Buffer (7.0 liters)

Maxi Kit (100 x 384):
384-Well Super Filter (100)
Microplate 384 well with cover (100) Microplate 384 well w/o cover (1)
Binding Buffer (7.0 liters)
Wash Buffer (13 liters)

Equipment Requirements

Microplate vacuum filter block
Multi-channel pipetting device (capable of 20-150 µl)
Multi-channel pipetting device (capable of 100 µl)
Microplate centrifuge with vacuum capabilities

384-Well PCR Purification Short Protocol (Steps 1-15)

1. Obtain a 384-well plate of PCR products concentrated to 20 µl per well.
2. Add 80 µl per well of Binding Buffer.
3. Mix the PCR sample and Binding Buffer by pipetting 10 times.
4. Transfer the 100 µl contents of each well to the Super Filter positioned on a standard vacuum block. Do not add the entire mixed contents all at once....allow Binding Buffer and PCR product to flow through gently.
5. Maintain a gentle vacuum to allow the entire 100 µl concentration of sample to flow through the Super Filter membrane to ensure proper DNA attachment.
6. Wash each well immediately with three (3) successive 100 µl volumes of 384-Well PCR Purification Kit™ 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 384 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 bottom 384 well microplate and its contents.
11. Transfer the Super Filter onto a second unused 384-well microplate.
12. Add 100 µl per well of 0.1X TE (pH = 8.0) or 100 µl per well of ddH_2O.
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.

384-Well PCR Purification Complete Protocol (1-15)

1. Amplify double-stranded DNA products by use of the polymerase chain reaction (PCR) under conditions of choice. Use 25-40 µl reaction volumes in a 384-well format. Following PCR amplification, reduce the volume to 20 µl per well by use of a 384-well microplate centrifuge equipped with vacuum capabilities. Approximately 30 minutes with heating is required to reduce a plate of 40 µl reactions to 20 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 20 µl per well of ddH_20 and re-suspend by pipetting up and down with a multi-channel pipetting device.

2. Add 80 µl of 384-Well PCR Purification Kit™ Binding Buffer to each 20 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 75 µl. Plugged pipet tips can be used to avoid contamination by aerosols generated during mixing. The pale blue color of the Binding Buffer can be used as a visual aid to monitor mixing.

4. Position a 384 Well Super Filter on a standard vacuum block. Slowly transfer the 100 µl contents of each well to the Super Filter allowing the reagent to gently flow through. Add the PCR product and buffer to the Super Filter slowly to keep the wells from overflowing. This transfer step is most efficiently made with a multi-channel pipetting device set to 125 µl. A pipette setting of 125 µl (instead of 100 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. Maintain the 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 or high capacity vacuum manifold. 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 100 µl aliquots of 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. 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 or high capacity vacuum manifold. The DNA remains bound to the Super Filter membrane. The pale blue appearance will 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 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 unused 384-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 384-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 384-well plate contains the PCR product bound to the membrane.

11. Place the Super Filter onto a second unused 384-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) or 100 ul per well of ddH_2O with a multi-channel pipetting device. For maximal DNA recovery, be sure to add the 0.1X TE or 100 ul per well of ddH_2O directly onto the surface of the Super Filter membrane. The mild

elution buffer (1 mM Tris-HCl, 0.1 mM EDTA) is used to minimize interference of the buffer in downstream applications. Warning...prolonged storage of DNA in ddH_2O may lead to degradation of the DNA product.

13. Re-hydrate the Super Filter membrane for 1 minute at ambient temperature. Incubation periods in excess of 1 minute do not increase DNA yield. Shorter incubations can reduce yield.

14. 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 384-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.

15. 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 10-20 ng per µl in ~80 µl 0.1X TE. The yield should be >90%. Evaporate the samples to dryness by vacuum centrifigation, re-suspend in buffer of choice and seal the samples for future use with the microplate cover.

384-Well PCR Purification Kit Protocol Modified for Centrifuge

1. Obtain a 384-well plate of PCR products concentrated to 18 µl per well.

2. Add 72 µl per well of Binding Buffer (note that total vol is 90 µl) The volume is reduced slightly as no vacuum is being used and the wells have a tendency to spill over and cross-contaminate when 100 µl is used.

3. Mix the PCR sample and Binding Buffer by pipetting 10 times.

4. Transfer the 90µl contents of each well to a Super Filter positioned on top of a tip box cover, placed on a microplate centrifuge rotor such as the Beckman S2096. Important: balance the centrifuge with a second microplate/tip-box cover. Equalize the weight between the two by adding dH_2O to the balance plate tip-box cover so that it is equal to the volume of sample loaded into the filter plate (also keeps the air inside the centrifuge humid)

5. Centrifuge at 500 x g at ambient temperature for a total of 30 seconds which includes the time taken for the centrifuge to reach speed. Tip out the waste from the Super Filter catch tray to keep the two plates balanced.

6. Wash each well immediately with three (3) successive 100 µl volumes of Wash Buffer using 500 x g/30 seconds centrifugation emptying the catch tray each wash.

7. Centrifuge the plates 500 x g for 10 minutes at ambient temperature to remove residual Wash Buffer.

8. Transfer the Super Filter onto an unused 384-well microplate.

9. Add 100 µl per well of 0.1X TE (pH = 8.0) or 100 µl per well of ddH2O.

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

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

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

Troubleshooting Tips

The 384-Well 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 through the 384-well 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.