UltraClean®-htp
96 Well PCR Clean-Up Kit

Catalog No.	Quantity	Total Isolations
12596-4	4 x 96 Preps	384

Instruction Manual

Please recycle

Version: 09122013

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Introduction

The UltraClean®-htp PCR Clean-Up Kit is designed to purify PCR products directly from a PCR or enzyme reaction in just 3 minutes without running an agarose gel. If you sequence your PCR reactions or have applications where efficient removal of PCR primers is critical, this kit is your solution. All reagents are optimized to remove primers, dNTPs and reaction components while purifying PCR reaction products in the size range of 60 bp to 10 kb.

Protocol Overview

This kit is designed to isolate plasmid DNA in 96 well plates. With the addition of binding buffer, a silica membrane spin filter plate is used to selectively bind the PCR or reaction product. Unwanted reaction components are passed through the filter plate by centrifugation. The desired product is then washed and recovered from the spin filter plate in certified DNA-free Tris buffer. The resulting DNA can be used for any downstream application.

Specifications:

DNA Size range: 60 bp - 10 kb
Binding capacity of Spin Plate: 20 µg
Final volume of DNA: 100 µl
Recovery rates: 80-100%

This kit is for research purposes only. Not for diagnostic use.

Other Related Products	Catalog No.	Quantity
100 bp DNA Ladder	17100-40	40 µg
UltraClean® 15 DNA Purification Kit	12100-300	300 preps
UltraClean® GelSpin® DNA Extraction Kit	12400-50 12400-100 12400-250	50 preps 100 preps 250 preps

Equipment Required

Centrifuge capable of spinning two 96 Well blocks stacked (13 cm x 8.5 cm x 6.1 cm) at 4500 x *g*

Note: If you have a centrifuge with a maximum speed less than 4500 x *g* see the Hints and Troubleshooting Guide.

Multi-channel Pipettor (volumes required 10 µl - 1000 µl)

Kit Contents

	Kit Catalog# 12596-4	
Component	**Catalog #**	**Amount**
SpinBind	12596-4-1	211 ml
SpinClean®	12596-4-2	4 x 30 ml
Elution Buffer	12596-4-3	43 ml
Spin Plate	12596-4-SP	4 plates
0.5 ml Collection Plates	12596-4-CP	12 plates
Sealing Tapes	12596-4-ST	4 tapes
Centrifuge Tapes	12596-4-CT	12 tapes
Microplates	12596-4-MP	4 plates
Elution Sealing Mats	12596-4-ESM	4 mats

Kit Storage

Kit reagents and components should be stored at room temperature (15-30°C).

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: SpinClean® contains ethanol. It is flammable.

IMPORTANT NOTE FOR USE: Shake to mix the SpinBind before use.

Centrifuge Protocol

Please wear gloves at all times

Before you start:

There are several things that will make this protocol more efficient to use.

- First, be sure to measure the centrifuge and rotor you plan to use and be sure they will accommodate the plates used in this kit. For this Centrifuge Protocol it is best to stack a Spin Plate on top of a 0.5 ml Collection Plate. Place this in the plate holder rotor. DO NOT start the centrifuge or possible injury or centrifuge damage may occur. Turn the centrifuge by hand slowly and be sure the stacked plates will clear the rotor and centrifuge.
- Make sure you have a multi-channel pipettor that can accommodate all the required volumes (10 µl - 1000 µl).
- This protocol assumes you will be processing 192 samples (2-96 well preps). If you plan to process less than this number, divide your samples between two plates evenly so that you always have a balance (see Hints and Troubleshooting Guide).

Protocol

1. Shake to mix the SpinBind before use. Add 5 volumes of SpinBind to your PCR reaction. Example: add 500 µl to a 100 µl PCR reaction. (If your PCR reaction and SpinBind volume is too large to fit in the PCR plate, you can use a 0.5 ml Collection Plate.)
2. Mix well by pipetting. If an oil overlay was used, you will now have two layers. The top layer is oil.
3. Place a 0.5 ml Collection Plate under a Spin Plate.
4. Transfer PCR/SpinBind mixture to the wells of the Spin Plate, while avoiding the transfer of oil.
   NOTE: If you do not use all the wells of the Spin Plate, any unused wells may be used at a later time.
5. Seal the wells with a piece of Centrifuge Tape.
6. Centrifuge the Spin Plate/ Collection Plate at 4500 x *g* for 3 minutes.
7. Remove the Spin Plate and discard the liquid flow-through from the 0.5 ml Collection Plate by inverting into an appropriate waste receptacle.
8. Remove Centrifuge Tape and replace the Spin Plate in the same 0.5 ml Collection Plate.
9. Add 300 µl SpinClean® to the wells of the Spin Plate. Seal Spin Plate with new piece of Centrifuge Tape.
10. Centrifuge at 4500 x *g* for 3 minutes.
11. Remove the Spin Plate and discard the liquid flow-through from the Collection Plate by inverting into waste receptacle.
12. Replace the Spin Plate in the same 0.5 ml Collection Plate.
13. Centrifuge again at 4500 x *g* for 6 minutes.
14. Carefully transfer the Spin Plate to a Microplate.
15. Remove Centrifuge Tape.
16. Allow to air dry for 10 minutes at room temperature.
17. Add 100 µl of Elution Buffer (10mM Tris) provided or sterile water directly onto the center of the white spin filter membrane of the Spin Plate. The choice of using Tris or water at this point will not affect yield. DNA is more stable for storage in Tris.
18. Seal the Spin Plate with new Centrifuge Tape and centrifuge for 3 minutes at 4500 x *g*.
19. Remove the Spin Plate from the Microplate. Purified DNA is now in the Microplate. Seal the Microplate with Elution Sealing Mat provided. The DNA will be free of all reaction components such as primers, enzyme, salt, and dNTP's. Store DNA at -20°C. DNA is now ready to use.

Thank you for choosing the UltraClean®-htp 96 Well PCR Clean-Up Kit.

Hints and Troubleshooting Guide

If 96 Samples are not Processed

If you have less than 96 samples to process, the entire Spin Plate will not be used. You will need to follow these steps to process the samples through the Spin Plate:

1. Seal entire Spin Plate with Sealing Tape.
2. Using a scalpel blade cut out only the wells containing the PCR/SpinBind mixture.
3. This Sealing Tape is to be kept on the unused wells throughout the rest of the protocol.

Centrifuge with a Maximum Speed Less Than 4500 x g

Multiply the protocol time and speed to determine total x *g*. Divide the total by the maximum speed of your centrifuge (round up if necessary). This will be the number of minutes your centrifuge will need to run to achieve the appropriate overall force.

Example: 10 minutes at 4500 x *g* = 45000.
If your centrifuge has a maximum speed of 2500 x *g*, divide 45000 ÷ 2500 = 18 minutes of centrifugation.

Concentrating the DNA

Your final volume will be 100 µl. If this is too dilute for your purposes, add 4 µl of 5M NaCl and mix. Then add 200 µl of 100% cold ethanol. Mix. Centrifuge at 10,000 x *g* for 5 minutes. Decant all liquid. Dry residual ethanol in a speed vac or desiccator or ambient air. Resuspend precipitated DNA in desired volume.

DNA Floats Out of Well When Loaded on a Gel

Residual SpinClean® in the final sample. Prevent this by being careful in step 14 of centrifuge protocol. Ethanol precipitate to remove residues of SpinClean®. (See procedure for Concentrating the DNA above.)

Low Recovery

Low recoveries can be due to not mixing SpinBind well with your sample in step 2. Incomplete removal of SpinClean® can also reduce yields. Make sure your centrifuge is spinning at a minimum of 2500 x *g*.

Enzyme Reactions Inhibited

If you chose to elute in a buffer containing EDTA, you may see inhibition of subsequent enzymatic reactions. Re-purify the sample with this kit and use the Tris solution Elution Buffer provided for the elution step.

Contact Information

Technical Support:
Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: technical@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

Ordering Information:
Direct: Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: orders@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

For the distributor nearest you, visit our web site at www.mobio.com/distributors

Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com

Description	Catalog No.	Quantity
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps
UltraClean® GelSpin® DNA Extraction Kit	12400-50 12400-100 12400-250	50 preps 100 preps 250 preps
UltraClean® 15 DNA Purification Kit	12100-300	300 preps
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
Dye Dots®	15020-10 15020-20	10 plates 20 plates