



UltraClean-htp™ 96 Well PCR Clean-up™ Kit

(4) 96 Well Preps (384 Preps)
Catalog #12596-4

Instruction Manual

Introduction

This is a spin filter format kit for purifying DNA from PCR reactions, labeling reactions, and other enzyme reactions in 96 well blocks. This high throughput Spin Plate method kit yields DNA ready for any application.

Precautions

Please wear gloves when using this product. Avoid all skin contact with reagents in this kit. 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 on our web site at www.mobio.com. Reagents labeled flammable should be kept away from open flames and fire.

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

Equipment required for centrifuge protocol:

Centrifuge capable of spinning 96 Well blocks stacked (13 cm x 8 cm x 5.5 cm) at 2500 x g.
Pipettor (volumes required 50 µl - 500 µl).

Specifications:

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

Kit Contents: (4) 96 Well Purifications

<u>Component</u>	<u>Amt.</u>	<u>Description</u>
SpinBind	85 ml	Guanidine HCl/ Isopropanol
SpinClean	4 x 30 ml	Ethanol solution (Flammable)
Elution buffer	43 ml	10mM Tris pH 8.0(DNA-Free)
Spin Plate	4	96 Well Spin Plates
0.5 ml Collection Plates	12	0.5 ml 96 Well Plates
Sealing Tape	4	Tape to seal unused wells
Centrifuge Tape	12	Tape for sealing Binding Plates
Microplate	4	96 Well microplate for elution
Elution Sealing Mat	4	Sealing Mat for Microplate

Kit Storage

Room temperature for 1 year.

Precautions: Wear gloves. Avoid contact with all reagents

If eye or skin contact occurs, wash thoroughly with water.

Version: 06262006

Technical information: Toll free 1-800-606-6246, or 1-760-929-9911 email: technical@mobio.com



Centrifuge Protocol

Before you start: Please see precautions on previous page.

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 μ l- 1000 μ l).
- ◆ 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 section.

Please wear gloves at all times

1. Add 2 volumes of SpinBind to your PCR reaction. Example: add 200 μ l to a 100 μ l 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 2500 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 μ l SpinClean to the wells of the Spin Plate. Seal Spin Plate with new piece of Centrifuge Tape.
10. Centrifuge at 2500 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 2500 x g for 3 minutes.
14. Carefully transfer the Spin Plate to a Microplate.
15. Remove Centrifuge Tape.
16. Add 100 μ l of Elution buffer (10mM Tris) solution 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.
17. Seal the Spin Plate with new Centrifuge Tape, and centrifuge for 5 minutes at 2500 x g.
18. 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™ 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.

Concentrating the DNA

Your final volume will be 100 μ l. If this is too dilute for your purposes, add 4 μ l of 5M NaCl and mix. Then add 200 μ l 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.



Other UltraClean™ Kits available from MO BIO Laboratories, Inc.

<u>Kit description</u>	<u>Cat. number</u>
Plasmid Prep Kits	
6 minute Mini Plasmid Prep Kit (100 preps)	12300-100
6 minute Mini Plasmid Prep Kit (250 preps)	12300-250
25-50 ml Plasmid Prep Kit (20 preps)	12700-20
25-50 ml Plasmid Prep Kit (50 preps)	12700-50
250-500 ml Plasmid Prep Kit (10 preps)	12600-10
250-500 ml Plasmid Prep Kit (20 preps)	12600-20
Endotoxin-Free Plasmid Prep Kits	
Endotoxin-free Mini Prep Kit (100 preps)	12311-100
Endotoxin-free Mini Prep Kit (250 preps)	12311-250
Endotoxin-free Midi Prep Kit (10 preps)	12711-10
Endotoxin-free Maxi Prep Kit (10 preps)	12611-10
DNA Purification Kits	
Agarose Gel DNA Purification Kit (300 preps)	12100-300
Agarose Gel-Spin DNA Purification (100 preps)	12400-100
Agarose Gel-Spin DNA Purification (250 preps)	12400-250
PCR Clean-Up Kit (100 preps)	12500-100
PCR Clean-Up Kit (250 preps)	12500-250
DNA Isolation Kits	
DNA Blood Isolation Kit (100 preps)	12000-100
DNA BloodSpin Kit (50 preps)	12200-50
DNA BloodSpin Kit (250 preps)	12200-250
Mega BloodSpin Kit (10 preps)	12210-10
Soil DNA Isolation Kit (50 preps)	12800-50
Soil DNA Isolation Kit (100 preps)	12800-100
Soil DNA Mega Prep Kit (10 preps)	12900-10
Fecal DNA Isolation Kit (50 preps)	12811-50
Fecal DNA Isolation Kit (100 preps)	12811-100
Microbial DNA Isolation Kit (50 preps)	12224-50
Microbial DNA Isolation Kit (250 preps)	12224-250
Plant DNA Isolation Kit (50 preps)	13000-50
Plant DNA Isolation Kit (250 preps)	13000-250
Tissue DNA Isolation Kit (50 preps)	12334-50
Tissue DNA Isolation Kit (250 preps)	12334-250
Water DNA Isolation Kit (10 preps)	14800-10
Water DNA Isolation Kit (25 preps)	14800-25
Forensic DNA Kit- Single prep format (10 preps)	14000-10
Forensic DNA Kit- Single prep format (20 preps)	14000-20
RNA Isolation Kits	
Tissue RNA Isolation Kit (50 preps)	15000-50
Tissue RNA Isolation Kit (250 preps)	15000-250
Plant RNA Isolation Kit (20 preps)	13300-20
Plant RNA Isolation Kit (50 preps)	13300-50
Microbial RNA Isolation Kit (50 preps)	15800-50
Microbial RNA Isolation Kit (250 preps)	15800-250
Growth Media	
TB DRY (1 kg) Terrific Broth powder	12105-1
LB (1 kg) LB powder (Miller)	12106-1
LB Agar (1 kg) LB Agar Powder (Miller)	12107-1

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