UltraClean® Soil DNA Isolation Kit

Catalog No.	Quantity
12800-50	50 Preps
12800-100	100 Preps

Instruction Manual

New protocol instruction: *Shake Solution S3 to mix before using to ensure consistent results.*

F **Please recycle**

Version: 10232009

Table of Contents

Introduction

The MO BIO Laboratories UltraClean® Soil DNA Isolation Kit has become the method of choice among researchers around the world studying microbial organisms in soil. This kit will isolate cellular, PCR quality DNA from soil and ensures removal of humic acid inhibitors.

Protocol Overview

Soil samples are added to a bead beating tube containing beads, lysis solution, bead solution and Inhibitor Removal Solution. The principal is to lyse the microorganisms in the soil by a combination of heat, detergent, and mechanical force against specialized beads. The cellular components are lysed by mechanical action on a vortex. From the lysed cells, the released DNA is bound to a silica spin filter. The filter is washed, and the DNA is recovered in certified DNA-free Tris buffer.

Bead Beating Options

The UltraClean® Soil DNA Isolation Kit does not require homogenization using a Fastprep® or Precellys® instrument. However, if the microorganism of interest requires stronger homogenization than provided by a vortex, or if using a Bead Beater is desired, the UltraClean® Soil DNA Isolation Kit may be used in conjunction with these methods. A starting point for homogenization is a setting of 5 on the FastPrep® or 5000 RPM on the Precellys® for one pulse of 45 seconds using the Bead Solution Tubes provided in the kit. For fungus or other difficult species, a 10 minute 65°C heating step may be performed prior to bead beating. More than one pulse of bead beating, or harder beads may be used, however, keep in mind that the DNA integrity will decrease in size. Additional bead tubes are available using harder matrices for grinding (see below). Published references for using the UltraClean® Soil DNA Isolation Kit with a FastPrep® instrument are available from technical support.

High Throughput Options

MO BIO offers a vacuum based protocol for faster processing without centrifugation for the DNA binding and column washing steps for Spin Filters. The MO BIO PowerVac™ Manifold allows for processing of up to 20 spin filter preps at a time using the PowerVac™ Mini Spin Filter Adapters. For additional high throughput methods, the UltraClean®-htp 96 Well Soil DNA Isolation Kit is available for processing up to 2 x 96 samples using a centrifuge capable of spinning two 96 Well Blocks stacked (13 cm x 8 cm x 5.5 cm) at 2500 x *g.* For 96 well homogenization of soil, MO BIO offers the 96 Well Plate Shaker and Plate Adapter Set (MO BIO Catalog# 11996 & 11999, respectively.)

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

Other Related Products	Catalog No.	Quantity
UltraClean® Mega Soil DNA Isolation Kit	12900-10	10 preps
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps
Vortex Adapter, holds 24 (1.5-2.0 ml) tubes	13000-V1-24	1 unit
Ceramic Bead Tubes, 1.4 mm Ceramic Bead Tubes, 2.8 mm Glass Bead Tubes, 0.5 mm Glass Bead Tubes, 0.1mm	13113-50 13114-50 13116-50 13118-50	50 tubes 50 tubes 50 tubes 50 tubes
PowerVac™ Manifold	11991	1 manifold
PowerVac™ Mini System	11992	1 unit + 20 adapters
PowerVac™ Mini Spin Filter Adapters	11992-10 11992-20	10 adapters 20 adapters

UltraClean® Soil DNA Isolation Kit

Prepare Sample & Cell Lysis

- Add soil sample to UltraClean® Bead Tube
- Add Solution S1
- Add IRS Solution
- Attach to Vortex Adapter
- Vortex

Centrifuge

Protein Precipitation

- Add Solution S2
- Vortex
- Incubate at 4°C

Alternate Protocol for PowerVac™ Mini Spin Filter Adapter

Centrifuge

Bind DNA

- Add Solution S3
- Vortex
- Load into Spin Filter

Centrifuge

Vacuum

Wash

- Wash with Solution S4

Centrifuge

Centrifuge

Elute

- Elute with Solution S5

Elute

- Elute with Solution S5

Equipment Required

Microcentrifuge (10,000 x *g*)
Pipettor (volumes required 50 µl - 500 µl)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
Vortex Adapter (MO BIO Catalog# 13000-V1)

Reagents Required but not Included

100% ethanol (for the PowerVac™ Manifold protocol only)

Kit Contents

	Kit Catalog# 12800-50		Kit Catalog# 12800-100	
Component	**Catalog#**	**Amount**	**Catalog#**	**Amount**
Bead Solution Tubes (contain 550 µl solution)	12800-50-BST	50	12800-100-BST	100
Solution S1	12800-50-1	3.3 ml	12800-100-1	6.6 ml
IRS Solution	12800-50-IRS	11 ml	12800-100-IRS	22 ml
Solution S2	12800-50-2	14 ml	12800-100-2	27.5 ml
Solution S3	12800-50-3	72 ml	12800-100-3	143 ml
Solution S4	12800-50-4	16.5 ml	12800-100-4	30 ml
Solution S5	12800-50-5	3 ml	12800-100-5	6 ml
Spin Filter Units in 2 ml Tubes	12800-50-SF	50	12800-100-SF	100
2 ml Collection Tubes	12800-50-T	150	12800-100-T	300

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: Solution S4 contains ethanol. It is flammable. Do not use bleach to clean the inside of the PowerVac™ Manifold or to rinse the PowerVac™ Mini Spin Filter Adapters when attached to the manifold.

IMPORTANT NOTE FOR USE: Make sure the 2 ml Bead Solution Tubes rotate freely in your centrifuge without rubbing. Shake to mix Solution S3 before use.

Experienced User Protocol
(To maximize yields, follow the Alternative Protocol on the next page.)
Please wear gloves at all times

1. To the 2 ml **Bead Solution Tubes** provided, add 0.25 – 1 gram of soil sample. (For larger sample sizes up to 10 grams, we offer the UltraClean® Mega Soil DNA Isolation Kit, Catalog# 12900-10).
2. Gently vortex to mix.
3. **Check Solution S1.** If **Solution S1** is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60 µl of **Solution S1** and invert several times or vortex briefly.
5. Add 200 µl of **IRS Solution** (Inhibitor Removal Solution). This is only required if the DNA is to be used for PCR.
6. Secure **Bead Solution Tubes** horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See alternative lysis method for less DNA shearing). **Note:** If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5-10 minutes.
7. Make sure the 2 ml **Bead Solution Tubes** rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.
8. Transfer the supernatant to a clean **2 ml Collection Tube** (provided). **Note**: With 0.25 grams of soil and depending upon soil type, expect between 400 to 450 µl of supernatant. Supernatant may still contain some soil particles.
9. Add 250 µl of **Solution S2** and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
10. Centrifuge the tubes for 1 minute at 10,000 x *g.*
11. Avoiding the pellet, transfer 450 µl of supernatant to a clean **2 ml Collection Tube** (provided). ***(To transfer entire volume, follow alternative protocol steps 12 through 21.)***
12. Shake to mix Solution S3 before use. Add 900 µl of **Solution S3** to the supernatant and vortex for 5 seconds.
13. Load approximately 700 µl onto a **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute.
14. Discard the flow through and add the remaining supernatant to the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute. **Note**: A total of two loads for each sample processed are required.
15. Add 300 µl of **Solution S4** and centrifuge for 30 seconds at 10,000 x *g*.
16. Discard the flow through.
17. Centrifuge again at 10,000 x *g* for 1 minute.
18. Carefully place **Spin Filter** in a new clean **2 ml Collection Tube** (provided). Avoid splashing any **Solution S4** onto the **Spin Filter**.
19. Add 50 µl of **Solution S5** to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica spin filter membrane at this step (MO BIO Catalog# 17000-10).
20. Centrifuge at 10,000 x *g* for 30 seconds.
21. Discard the **Spin Filter**. DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20°C to -80°C). **Solution S5** contains no EDTA.

Thank you for choosing the UltraClean® Soil DNA Isolation Kit.

Alternative Protocol (For Maximum Yields)

Please wear gloves at all times

1. To the 2 ml **Bead Solution Tubes** provided, add 0.25 – 1 gram of soil sample. (For larger sample sizes up to 10 grams, we offer the UltraClean® Mega Soil DNA Isolation Kit, Catalog# 12900-10).
2. Gently vortex to mix.
3. **Check Solution S1.** If **Solution S1** is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60 µl of **Solution S1** and invert several times or vortex briefly.
5. Add 200 µl of **IRS Solution** (Inhibitor Removal Solution). This is only required if the DNA is to be used for PCR.
6. Secure **Bead Solution Tubes** horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See alternative lysis method for less DNA shearing). **Note:** If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5-10 minutes.
7. Make sure the 2 ml **Bead Solution Tubes** rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.
8. Transfer the supernatant to a clean **2 ml Collection Tube** (provided). **Note**: With 0.25 grams of soil and depending upon soil type, expect between 400 to 450 µl of supernatant. Supernatant may still contain some soil particles.
9. Add 250 µl of **Solution S2** and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
10. Centrifuge the tubes for 1 minute at 10,000 x *g.*
11. Avoiding the pellet, transfer entire volume of supernatant to a clean **2 ml Collection Tube** (provided).
12. Shake to mix Solution S3 before use. Add 1.3 ml of **Solution S3** to the supernatant and vortex for 5 seconds. **Note:** High volume of solution will touch the rim of the tube. Take care when handling tube.
13. Load approximately 700 µl onto a **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute.
14. Discard the flow through, add the remaining supernatant to the **Spin Filter**, and centrifuge at 10,000 x *g* for 1 minute. Repeat until all supernatant has passed through the **Spin Filter**. **Note**: A total of three loads for each sample processed are required.
15. Add 300 µl of **Solution S4** and centrifuge for 30 seconds at 10,000 x *g*.
16. Discard the flow through.
17. Centrifuge again at 10,000 x *g* for 1 minute.
18. Carefully place **Spin Filter** in a new clean **2 ml Collection Tube** (provided). Avoid splashing any **Solution S4** onto the **Spin Filter**.
19. Add 50 µl of **Solution S5** to the center of the white filter membrane.
20. Centrifuge at 10,000 x *g* for 30 seconds.
21. Discard the **Spin Filter**. DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20°C to -80°C). **Solution S5** contains no EDTA.

Thank you for choosing the UltraClean® Soil DNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)
Please wear gloves at all times

1. To the 2 ml **Bead Solution Tubes** provided, add 0.25 – 1 gram of soil sample. (For larger sample sizes up to 10 grams, we offer the UltraClean® Mega Soil DNA Isolation Kit, Catalog# 12900-10. For amounts of sample to process see Hints and Troubleshooting Guide).

   What's happening: *The soil sample or fecal sample has now been loaded into the Bead Tube. This is the first part of the lysis procedure. The Bead Solution is a buffer that will disperse the soil particles and begin to dissolve humic acids.*

2. Gently vortex to mix.

   What's happening: This step mixes the sample and Bead Solution.

3. **Check Solution S1**. If **Solution S1** is precipitated, heat solution to 60°C until dissolved before use.

   What's happening: Solution S1 contains SDS. If it gets cold, it will precipitate. Heating to 60°C will dissolve the SDS. The Solution S1 can be used while it is still warm.

4. Add 60 µl of **Solution S1** and invert several times or vortex briefly.

   What's happening: Solution S1 contains SDS. This is a detergent that aids in cell lysis. The detergent breaks down fatty acids and lipids associated with the cell membrane of several organisms.

5. Add 200 µl of **IRS Solution** (Inhibitor Removal Solution). This is only required if the DNA is to be used for PCR.

   What's happening: IRS is a proprietary reagent designed to precipitate humic acids and other PCR inhibitors. This precipitation step is required if the intended use of the DNA is for PCR. Humic acids are generally brown in color. They belong to a large group of organic compounds associated with most soils that are high in organic content.

6. Secure bead tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See alternative lysis method for less DNA shearing).
   Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5-10 minutes.

   What's happening: The method you use to secure tubes to the vortex is critical. We have designed the vortex adapter as a simple tool that keeps tubes tightly attached to the vortex. It should be noted that although you can attach tubes with tape, often the tape becomes loose and not all tubes will shake evenly or efficiently. This may lead to inconsistent results or lower yields. The use of the vortex adapter is highly recommended for maximum DNA yields.

   Mechanical lysis is introduced at this step. The protocol uses a combination of mechanical and chemical lysis. By randomly shaking the beads, they collide with one another and with microbial cells causing them to break open.

7. Make sure the 2 ml **Bead Solution Tubes** rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.

What's happening: Particulates including cell debris, soil, beads, and humic acids, will form a pellet at this point. DNA is in the liquid supernatant.

8. Transfer the supernatant to a clean **2 ml Collection Tube** (provided).

   ***Note**: With 0.25 grams of soil and depending upon soil type, expect between 400 to 450 µl of supernatant. Supernatant may still contain some soil particles.*

9. Add 250 µl of **Solution S2** and vortex for 5 seconds. Incubate at 4°C for 5 minutes.

   What's happening: Solution S2 contains a protein precipitation reagent. It is important to remove contaminating proteins that may reduce DNA purity and inhibit downstream applications for the DNA.

10. Centrifuge the tubes for 1 minute at 10,000 x *g.*

11. Avoiding the pellet, transfer entire volume of supernatant to a clean **2 ml Collection Tube** (provided).

    What's happening: The pellet at this point contains residues of humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

12. Shake to mix Solution S3 before use. Add 1.3 ml of **Solution S3** to the supernatant and vortex for 5 seconds.

    ***Note:** High volume of solution will touch the rim of the tube. Take care when handling tube.*

    What's happening: Solution S3 is a DNA binding salt solution. DNA binds to silica in the presence of high salt concentrations.

13. Load approximately 700 µl onto a **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute.

14. Discard the flow through, add the remaining supernatant to the **Spin Filter**, and centrifuge at 10,000 x *g* for 1 minute. Repeat until all supernatant has passed through the **Spin Filter**.

    ***Note**: A total of three loads for each sample processed are required.*

    What's happening: DNA is selectively bound to the silica membrane in the spin filter device. Almost all contaminants pass through the filter membrane, leaving only the desired DNA behind.

15. Add 300 µl of **Solution S4** and centrifuge for 30 seconds at 10,000 x *g*.

    What's happening: Solution S4 is an ethanol based wash solution used to further clean the DNA that is bound to the silica membrane in the spin filter. This wash solution removes residues of salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

    ***Note:** You can wash more than one time to further clean DNA if desired. In some cases where soils have very high humic acid content, it will be beneficial to repeat this wash step. There is 10% extra Solution S4 in the bottle for this purpose. Solution S4 is also sold separately (MO BIO Catalog# 12800-100-4).*

16. Discard the flow through from the **2 ml Collection Tube**.

    What's happening: This flow through is just waste containing ethanol wash solution and contaminants that did not bind to the silica spin filter membrane.

17. Centrifuge again at 10,000 x *g* for 1 minute.

    What's happening: This step removes residual Solution S4 (ethanol wash solution). It is critical to remove all traces of wash solution because it can interfere with down stream applications for the DNA.

18. Carefully place **Spin Filter** in a new clean **2 ml Collection Tube** (provided). Avoid splashing any **Solution S4** onto the **Spin Filter**.

    What's happening: Once again it is important to avoid any traces of the ethanol based wash solution.

19. Add 50 µl of **Solution S5** to the center of the white filter membrane.

    What's happening: Placing the Solution S5 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in more efficient release of the desired DNA.

20. Centrifuge at 10,000 x *g* or 30 seconds.

    What's happening: As the Solution S5 (elution buffer) passes through the silica membrane, DNA is released, and it flows through the membrane, and into the collection tube. The DNA is released because it can only bind to the silica spin filter membrane in the presence of salt. Solution S5 is 10mM Tris pH 8.0 and does not contain salt.

21. Discard the **Spin Filter**. DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20°C to -80°C). **Solution S5** contains no EDTA.

Thank you for choosing the UltraClean® Soil DNA Isolation Kit.

Vacuum Protocol using the PowerVac™ Manifold

Please wear gloves at all times

For each sample lysate, use one Spin Filter column. Keep the Spin Filter in the attached 2 ml Collection Tube and continue using the Collection Tube as a Spin Filter holder until needed for the Vacuum Manifold Protocol. Label each Collection Tube top and Spin Filter column to maintain sample identity. If the Spin Filter becomes clogged during the vacuum procedure, you can switch to the procedure for purification of the DNA by centrifugation.

You will need to provide 100% ethanol for step 4 of this protocol

1. For each prep, attach one aluminum **PowerVac™ Mini Spin Filter Adapter** (MO BIO Catalog# 11992-10 or 11992-20) into the Luer-Lok® fitting of one port in the manifold. Gently press a Spin Filter column into the PowerVac™ Mini Spin Filter Adapter until snugly in place. Ensure that all unused ports of the vacuum manifold are closed.
   Note: Aluminum PowerVac™ Mini Spin Filter Adapters are reusable.

2. Transfer 650 µl of prepared sample lysate (from step 12) to the **Spin Filter column**.

3. Turn on the vacuum source and open the stopcock of the port. Hold the tube in place when opening the stopcock to keep the spin filter steady. Allow the lysate to pass through the **Spin Filter column**. After the lysate has passed through the column completely, load again with the next 650 µl of lysate. Continue until all of the lysate has been loaded onto the **Spin Filter column**. Close the one-way Luer-Lok® stopcock of that port.
   Note: If Spin Filter Columns are filtering slowly, close the ports to samples that have completed filtering to increase the pressure to the other columns.

4. Load 800 µl of 100% ethanol into the Spin Filter so that it completely fills the column. Open the stopcock while holding the column steady. Allow the ethanol to pass through the column completely. Close the stopcock.

5. Add 300 µl of **Solution S4** to each Spin Filter. Open the Luer-Lok® stopcock and apply a vacuum until **Solution S4** has passed through the Spin Filter completely. Continue to pull a vacuum for another minute to dry the membrane. Close each port.

6. Turn off the vacuum source and open an unused port to vent the manifold. If all 20 ports are in use, break the vacuum at the source. Make certain that all vacuum pressure is released before performing the next step. It is important to turn off the vacuum at the source to prevent backflow into the columns.

7. Remove the **Spin Filter column** and place in the original labeled **2 ml Collection Tube**. Place into the centrifuge and spin at 13,000 × *g* for 1 minute to completely dry the membrane.

8. Transfer the **Spin Filter column** to a new **2 ml Collection Tube** and add 50 µl of **Solution S5** to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica **Spin Filter** membrane at this step (MO BIO Catalog # 17000-10).

9. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.

10. Discard the **Spin Filter column**. The DNA in the tube is now ready for any downstream application. No further steps are required.

    We recommend storing DNA frozen (-20° to -80°C). **Solution S5** contains no EDTA. To concentrate the DNA see the Hints & Troubleshooting Guide.

Thank you for choosing the UltraClean® Soil DNA Isolation Kit.

Hints and Troubleshooting Guide

Amount of Soil to Process
Depending on soil type, usually 0.25 -1 gram works well. Typically, only 0.25 g of the more absorbent soil types, such as potting soils, can be processed. For wet soils, see "Wet Soil Sample" below.

Wet Soil Sample
If soil sample is high in water content remove contents from bead tube (beads and solution) and set aside. Add soil sample to bead tube and centrifuge for 30 seconds at 10,000 x *g*. Remove as much liquid as possible with a pipet tip. Add beads and bead solution back to bead tube and follow protocol starting at step 2.

If DNA Does Not Amplify
This is due to high humic acid content in soil sample. If the humic acid content is sample is high, you can do the following:

- Diluting template DNA may also work because this will also dilute the inhibitors of the reaction.
- Perform two to three washes of Solution S4 in steps 15 through 18.
- Dilute the elution three fold and add two volumes of Solution S3. Run through spin filter, wash and elute.
- Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. An excess amount of DNA will also inhibit a PCR reaction.
- If DNA will still not amplify after trying the steps above, then PCR optimization may be needed.

Elution Sample Still Brown
This is due to high humic acid content in soil sample. If the humic acid content is sample is high, you can do two to three washes of Solution S4 in steps 15 through 18. If elution solution is still brown, dilute the elution three fold and add two volumes of Solution S3. Run through spin filter, wash and elute.

Alternative Lysis Method
After adding Solution S1, vortex 3-4 seconds. Add the IRS Solution, vortex 3-4 seconds then heat to 70°C for 5 minutes. Vortex 3-4 seconds. Heat another 5 minutes. Vortex 3-4 seconds. This alternative procedure will reduce shearing but may reduce yield.

Concentrating the DNA
Your final volume will be 50 µl. If this is too dilute for your purposes, add 2 µl of 5 M NaCl and mix. Add 100 µl of 100% cold ethanol and mix. Centrifuge at 10,000 x *g* for 5 minutes. Decant all liquid. Dry residual ethanol in a speed vac, desiccator, or air dry. Resuspend precipitated DNA in desired volume.

DNA Floats Out of Well When Loaded on a Gel
You may have inadvertently transferred some residual Solution S4 into the final sample. Prevent this by being careful in step 18 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation is the best way to remove Solution S4 residue. (See *Concentrating the DNA* above)

Storing DNA
DNA is eluted in Solution S5 (10 mM Tris) and must be stored at -20°C or it may degrade over time. DNA can be eluted in TE but the EDTA may inhibit reactions such as PCR and automated sequencing.

Hints & Troubleshooting Guide cont.

Cells are Difficult to Lyse

If cells are difficult to lyse, a 10 minute incubation at 70°C, after adding Solution S1, can be performed. Follow by continuing with protocol step 5.

Cleaning of the PowerVac™ Mini Spin Filter Adapters

It is recommended to rinse the PowerVac™ Mini Spin Filter Adapters promptly after use to avoid salt build up. To clean the PowerVac™ Mini Spin Filter Adapters, rinse each adapter with DI water followed by 70% ethanol and flush into the manifold base. Alternatively, remove the adapters and wash in laboratory detergent and DI water. PowerVac™ Mini Spin Filter Adapters may be autoclaved.

Do not use bleach to clean the PowerVac™ Mini Spin Filter Adapters while attached to the PowerVac™ Manifold. Bleach should never be mixed with solutions containing guanidine and should not be used to clean the PowerVac™ Manifold. For more information on cleaning the PowerVac™ Manifold, please refer to the PowerVac™ Manifold manual.

Contact Information

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

Other Quality Products Available from MO BIO Laboratories, Inc.

For more product and detailed information go to www.mobio.com/catalog-request to request a catalog.

DNA Purification and Gel Extraction	Catalog No.	Quantity
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
UltraClean® 15 DNA Purification Kit	12100-300	300 preps
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps
UltraClean®-htp 96 Well PCR Clean-Up Kit	12596-4 12596-12	4 x 96 preps 12 x 96 preps
UltraClean® GelSpin® DNA Extraction Kit	12400-50 12400-100 12400-250	50 preps 100 preps 250 preps
Plasmid DNA Isolation	**Catalog No.**	**Quantity**
UltraClean® 6 Minute Mini Plasmid Prep Kit	12300-50 12300-100 12300-250	50 preps 100 preps 250 preps
UltraClean® Standard Mini Plasmid Prep Kit	12301-50 12301-100 12301-250	50 preps 100 preps 250 preps
UltraClean®-htp 96 Well Plasmid Prep Kit	12396-4 12396-12	4 x 96 preps 12 x 96 preps
UltraClean® Midi Plasmid Prep Kit	12700-20 12700-50	20 preps 50 preps
UltraClean® Maxi Plasmid Prep Kit	12600-10 12600-20	10 preps 20 preps
UltraClean® Endotoxin-Free Mini Plasmid Prep Kit	12311-100 12311-250	100 preps 250 preps
UltraClean® Endotoxin-Free Midi Plasmid Prep Kit	12711-10	10 preps
UltraClean® Endotoxin-Free Maxi Plasmid Prep Kit	12611-10	10 preps
UltraClean® Endotoxin Removal Kit	12615	1 kit
UltraClean® Endotoxin-Free Ethanol Precipitation Kit	12616	1 kit
UltraClean® Endotoxin Removal Reagent	12625-25	25 ml
Endotoxin-Free Sodium Chloride	12626-15	15 ml
Endotoxin-Free Centrifuge Tubes	12617-100 12618-50 12619-25	100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes
RNA Isolation	**Catalog No.**	**Quantity**
LifeGuard™ Soil Stabilization Solution	12868-10 12868-100 12868-1000 12868-7500	10 ml 100 ml 1 L 7.5 L
On-Spin Column DNase I Kit (RNase-Free)	15100-50	50 preps
BiOstic® Stabilized Blood RNA Isolation Kit	12231-20 12231-50 12231-100	20 preps 50 preps 100 preps
BiOstic® Blood Total RNA Isolation Kit	12230-20 12230-50	20 preps 50 preps
RNA PowerSoil® DNA Elution Accessory Kit	12867-25	25 preps
RNA PowerSoil® Total RNA Isolation Kit	12866-25	25 preps
UltraClean® Microbial RNA Isolation Kit	15800-50 15800-250	50 preps 250 preps
UltraClean® Tissue & Cells RNA Isolation Kit	15000-50 15000-250	50 preps 250 preps

RNA Isolation …*Continued*	Catalog No.	Quantity
UltraClean® Plant RNA Isolation Kit	13300-20 13300-50	20 preps 50 preps
Genomic DNA Isolation	**Catalog No.**	**Quantity**
PowerFood™ Microbial DNA Isolation Kit	21000-50 21000-100	50 preps 100 preps
BiOstic® Bacteremia DNA Isolation Kit	12240-50	50 preps
BiOstic® FFPE Tissue DNA Isolation Kit	12250-50	50 preps
BiOstic® Paraffin Removal Reagent	12251-50	2 x 25 ml
PowerMax® Soil DNA Isolation Kit	12988-10	10 preps
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
PowerSoil®-htp 96 Well Soil DNA Isolation Kit	12955-4 12955-12	4 x 96 preps 12 x 96 preps
UltraClean® Soil DNA Isolation Kit	12800-50 12800-100	50 preps 100 preps
UltraClean®-htp 96 Well Soil DNA Isolation Kit	12896-4 12896-12	4 x 96 preps 12 x 96 preps
UltraClean® Mega Soil DNA Isolation Kit	12900-10	10 preps
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
UltraClean® Fecal DNA Isolation Kit	12811-50 12811-100	50 preps 100 preps
PowerMicrobial® Midi DNA Isolation Kit	12225-25	25 preps
PowerMicrobial® Maxi DNA Isolation Kit	12226-25	25 preps
UltraClean® Microbial DNA Isolation Kit	12224-50 12224-250	50 preps 250 preps
UltraClean®-htp 96 Well Microbial DNA Isolation Kit	10196-4 10196-12	4 x 96 preps 12 x 96 preps
PowerPlant® DNA Isolation Kit	13200-50 13200-100	50 preps 100 preps
UltraClean® Plant DNA Isolation Kit	13000-50 13000-250	50 preps 250 preps
UltraClean®-htp 96 Well Plant DNA Isolation Kit	13096-4 13096-12	4 x 96 preps 12 x 96 preps
UltraClean® Tissue & Cells DNA Isolation Kit	12334-50 12334-250	50 preps 250 preps
UltraClean®-htp 96 Well Tissue DNA Isolation Kit	12996-4 12996-12	4 x 96 preps 12 x 96 preps
UltraClean® Blood DNA Isolation Kit (Non-Spin)	12000-100	100 preps
UltraClean® Blood DNA Isolation Kit (Processes 1,000 ml of Blood)	12000-1000	1 kit
UltraClean® Blood DNA Isolation Kit Plus RNase (Processes 1,000 ml of Blood)	12002-1000	1 kit
UltraClean® BloodSpin® DNA Isolation Kit	12200-50 12200-250	50 preps 250 preps
UltraClean®-htp 96 Well BloodSpin® DNA Isolation Kit	12296-4 12296-12	4 x 96 preps 12 x 96 preps

Other Quality Products Available from MO BIO Laboratories, Inc.

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Genomic DNA Isolation *...Continued*	Catalog No.	Quantity
UltraClean® Forensic DNA Isolation Kit	14000-10 14000-20	10 isolations 20 isolations
PowerWater® DNA Isolation Kit	 14900-50-NF 14900-50-22 14900-50-45 14900-100-NF 14900-100-22 14900-100-45	50 preps (No filters) (0.22 µm) (0.45 µm) 100 preps (No filters) (0.22 µm) (0.45 µm)
RapidWater™ DNA Isolation Kit	 14810-50-NF 14810-50-22 14810-50-45 14810-100-NF 14810-100-22 14810-100-45	50 preps (No filters) (0.22 µm) (0.45 µm) 100 preps (No filters) (0.22 µm) (0.45 µm)
UltraClean® Water DNA Isolation Kit (0.45µm filters)	14800-10 14800-25	10 preps 25 preps
UltraClean® Water DNA Isolation Kit (0.22 µm filters)	14880-10 14880-25	10 preps 25 preps
UltraClean® Water DNA Isolation Kit (No filters)	14800-10-NF 14800-25-NF	10 preps 25 preps
Microbiological Culture Media	**Catalog No.**	**Quantity**
TB DRY® Powder Growth Media	12105-05 12105-1 12105-5	500 g 1 kg 5 kg
LB Broth Powder Growth Media, pH 7	12106-05 12106-1 12106-5	500 g 1 kg 5 kg
LB Agar Powder Growth Media, pH 7	12107-05 12107-1 12107-5	500 g 1 kg 5 kg
LB Broth (Lennox) Powder Growth Media, pH 7	12108-05 12108-1 12108-5	500 g 1 kg 5 kg
LB Agar (Lennox) Powder Growth Media, pH 7	12109-05 12109-1 12109-5	500 g 1 kg 5 kg
Soybean-Casein Digest Medium (TSB), USP	12114-05 12114-1 12114-5	500 g 1 kg 5 kg
Soybean-Casein Digest Agar Medium (TSA), USP	12115-05 12115-1 12115-5	500 g 1 kg 5 kg
Yeast Extract	12110-05 12110-1 12110-5	500 g 1 kg 5 kg
Tryptone	12111-05 12111-1 12111-5	500 g 1 kg 5 kg
Agar, Bacteriological Grade	12112-05 12112-1 12112-5	500 g 1 kg 5 kg
Other Reagents and Lab Accessories	**Catalog No.**	**Quantity**
20 bp DNA Ladder	17020-40	40 µg

Other Reagents and Lab Accessories...*Continued*	Catalog No.	Quantity
100 bp DNA Ladder	17100-40	40 µg
1 kb DNA Ladder	17200-100	100 µg
UltraClean® Agarose, Molecular Biology Grade	15003-50 15003-100 15003-500 15003-1000	50 g 100 g 500 g 1 kg
UltraClean® MS-8 Agarose	15515-50 15515-100 15515-500	50 g 100 g 500 g
UltraClean® Forensic Agarose	15505-50 15505-100 15505-500	50 g 100 g 500 g
UltraClean® Low Melt Agarose	15005-50 15005-100 15005-500	50 g 100 g 500 g
UltraClean® Low Melt Sieve Agarose	15004-50 15004-100 15004-500	50 g 100 g 500 g
Ethidium Bromide Solution	15006-1 15006-10	1 ml 10 ml
Ethidium Bromide Destaining Tea Bags	15007-25	25 bags
Bromophenol Blue Gel Loading Buffer	15008-1 15008-5	1 ml 5 x 1 ml
Bromophenol Blue/Xylene Cyanol Gel Loading Buffer	15009-1 15009-5	1 ml 5 x 1 ml
TAE Buffer, 50X (Tris-acetate-EDTA)	15001-100 15001-500 15001-1000	100 ml 500 ml 1 liter
TBE Buffer, 10X (Tris-borate-EDTA)	15002-100 15002-500 15002-1000	100 ml 500 ml 1 liter
RNase-Free Gloves	1555-XS 1555-S 1555-M 1555-L	bag of 100 bag of 100 bag of 100 bag of 100
UltraClean® Lab Cleaner	12095-250 12095-500 12095-1000	250 ml squeeze bottle 500 ml spray bottle 1 liter bottle
OmniTaq™ DNA Polymerase Enzyme	1224-250	250 reactions (10 U/µl)
OmniTaq™ DNA Polymerase 2x Master Mix	1226-250	250 reactions (5 x 1.25 ml/tube)
Omni KlenTaq™ DNA Polymerase Enzyme	1225-250	250 reactions (25 U/µl)
Omni KlenTaq™ DNA Polymerase 2x Master Mix	1227-250	250 reactions (5 x 1.25 ml/tube)

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Other Reagents and Lab Accessories…*Continued*	Catalog No.	Quantity
DNase (RNase-Free)	15600-5 15601-100	5 mg 2500 units
Proteinase K	1223-100 1222-2	100 mg 2 ml (20 mg/ml)
Ribonuclease A (25 mg/ml)	1202-1 1202-5	1 ml 5 ml
PCR Water	17000-1 17000-5 17000-10 17000-11	1 ml 5 x 1 ml 10 x 1 ml 10 ml bottle
Molecular Biology Grade Water	17012-200 17012-5200	200 ml 5 x 200 ml
DEPC Treated Water	17011-200 17011-5200	200 ml 5 x 200 ml
Endotoxin-Free Water	17013-10 17013-50 17013-100 17013-500	10 ml 50 ml 100 ml 500 ml
Instrumentation and Accessories	**Catalog No.**	**Quantity**
BagMixer® 400 VW	23112	1 unit
BagFilter® 400 P	23113-500	Box of 500
BagPage® 400	23114-500	Box of 500
Precellys®24 Homogenizer, 120V	13112	1 unit
Ceramic Bead Tubes, 1.4 mm	13113-50	50 bead tubes
Ceramic Bead Tubes, 2.8 mm	13114-50	50 bead tubes
Glass Bead Tubes, 0.5 mm	13116-50	50 bead tubes
Glass Bead Tubes, 0.1 mm	13118-50	50 bead tubes
Metal Bead Tubes, 2.38 mm	13117-50	50 bead tubes
2.0 ml Tough Tubes with Cap	13119-500 13119-1000	500 1000
Carbide Bead Tubes, 0.25 mm	13121-50	50 x 0.5 ml tubes
Garnet Bead Tubes, 0.15 mm	13122-50	50 x 0.5 ml tubes
Garnet Bead Tubes, 0.70 mm	13123-50	50 x 2 ml tubes
Garnet + ¼ Ceramic 15 ml Bead Tubes, 0.70 mm	13134-50	50 tubes
Garnet + ¼ Ceramic 50 ml Bead Tubes, 0.70 mm `	13144-10 13144-50 13144-100 13144-500	10 tubes 50 tubes 100 tubes 500 tubes
Glass 15 ml Bead Tubes, 0.1 mm	13135-50	50 tubes

Instrumentation and Accessories… *Continued*	Catalog No.	Quantity
Glass 50 ml Bead Tubes, 0.1 mm	13145-10 13145-50 13145-100 13145-500	10 tubes 50 tubes 100 tubes 500 tubes
Glass 15 ml Bead Tubes, 1.0 mm	13136-50	50 tubes
Ceramic 15 ml Bead Tubes, 1.4 mm	13137-50	50 tubes
Ceramic 50 ml Bead Tubes, 1.4 mm	13147-10 13147-50	10 tubes 50 tubes
Metal 50 ml Bead Tubes, 2.38 mm	13149-10 13149-50	10 tubes 50 tubes
PowerMix 15 ml Bead Tubes	13138-50	50 tubes
PowerMix 50 ml Bead Tubes	13148-10 13148-50	10 tubes 50 tubes
2 ml Collection Tubes	1200-100-T 1200-150-T 1200-250-T	100 tubes 150 tubes 250 tubes
2 ml Screw Cap Tubes	12800-200-E	200 tubes & caps
15 ml Collection Tubes	12700-T	25 tubes
50 ml Centrifuge Tubes	12600-T	25 tubes
Spin Filters (in 1.9 ml tubes)	1200-50-SF 1200-100-SF 1200-250-SF	50 filters 100 filters 250 filters
Endotoxin-Free Centrifuge Tubes	12617-100 12618-50 12619-25	100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes
15 ml Midi Spin Filters	12700-SF	25 spin filters
Vortex-Genie® 2 Vortex (120V)	13111-V	1 unit
Vortex-Genie® 2 Vortex (220V)	13111-V-220	1 unit
Vortex Adapter, holds 12 (1.5-2.0 ml) tubes	13000-V1	1 unit
Vortex Adapter, holds 6 (5 ml) tubes	13000-V1-5	1 unit
Vortex Adapter, holds 4 (15 ml) tubes	13000-V1-15	1 unit
Vortex Adapter, holds 2 (50 ml) tubes	13000-V1-50	1 unit
Vortex Adapter, holds 24 (1.5-2.0 ml) tubes	13000-V1-24	1 unit
Power Supply w/Timer, (120V)	16023	1 unit
Power Supply w/Timer, (220V)	16023-220	1 unit
Polycarbonate Single-sided Comb	16005 16006 16007 16008	1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well

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Instrumentation and Accessories… *Continued*	Catalog No.	Quantity
Polycarbonate Dual-sided Comb	16013 16014 16015 16016	1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well
Teflon Single-sided Comb	16009 16010 16011 16012	1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well
Teflon Dual-sided Comb	16017 16018 16019 16020	1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well
Mini Horizontal Gel System	16001	1 each
Mini Horizontal Gel Caster, 3 place	16003	1 each
Mini Horizontal Gel Tray	16004	1 each
96 Well Plate Shaker (120V)	11996	1 unit

Instrumentation and Accessories… *Continued*	Catalog No.	Quantity
96 Well Plate Shaker (220V)	11996-220	1 unit
Plate Adapter Set	11999	1 set
Tube Adapter Set	11995	1 set
Vacuum Pump (120V)	11998	1 unit
Vacuum Pump (220V)	11998-220	1 unit
UltraVac™ Manifold	11997	1 unit