RNA PowerSoil®
Total RNA Isolation Kit

Catalog No.	Quantity
12866-25	25 Preps

Instruction Manual

New protocol instructions:

- (Steps 3-5) Phenol:chloroform:isoamyl alcohol (pH 6.5 – 8.0, [User supplied]) is now added before vortexing begins rather than being added after 5 minutes of vortexing.
- (Step 12) The incubation at Step 12 is recommended to be carried out at room temperature. The previous protocol instructions were to incubate at -20°C for 30 minutes. If you have previously used the -20°C incubation before and know that your soil type yields good results at that temperature, you may continue to follow that protocol.

Please recycle

Version: 08192016

Table of Contents

Introduction

MO BIO Laboratories' RNA PowerSoil® Total RNA Isolation Kit is designed to isolate total RNA from organisms found in soil. The properties of this kit permit consistent removal of humic substances, fulvic acids, and other RT-PCR inhibitors from RNA purified from soil. Diverse soil types, including compost, manure, estuary sediment, and other soil types high in organic content, have successfully provided intact and RT-PCR amplifiable RNA using this kit. The RNA PowerSoil® Total RNA Isolation Kit reliably provides RNA for experiments requiring qualitative and quantitative RT-PCR analysis.

For collection and transport of soils needed for RNA extraction, the LifeGuard™ Soil Preservation Solution is a specially formulated treatment that protects the viability of bacteria in soil while keeping them in stasis. RNase and DNase activity is prevented allowing accurate full length cDNA synthesis and 16S rRNA profiling on samples collected in the field. Microbial community profiles are maintained in soils stabilized with LifeGuard™ for up to 30 days at -20°C, 1 week at 4°C, and 3 days at room temperature. *We do not recommend the use of RNALater and RNAProtect for preservation of nucleic acids in soils.*

Protocol Overview

For use with up to 2 grams of soil, homogenization occurs in a 15 ml tube containing silica carbide beads, lysis buffers, phenol:chloroform:isoamyl alcohol pH 6.5-8, and IRS, to ensure a complete lysis of all microorganisms and neutralization of RNases. Cleared lysates are precipitated to concentrate the total nucleic acids and pellets are resuspended in a buffer optimized for binding to anion-exchange gravity flow columns. RNA is eluted using a high salt buffer and precipitated to obtain the final pure RNA resuspended in RNase-Free water. DNA may be purified separately from the RNA using an alternative high salt buffer provided in a kit called the RNA PowerSoil® DNA Elution Accessory Kit. Nucleic acids are ready to use in enzymatic applications.

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

Other Related Products	Catalog No.	Quantity
RNA PowerSoil® DNA Elution Accessory Kit	12867-25	25 preps
LifeGuard™ Soil Preservation Solution	12868-100 12868-1000	100 ml 1000 ml
DNase Max™ Kit	15200-50	50 preps

IMPORTANT: This kit requires user provided phenol:chloroform:isoamyl alcohol (25:24:1, pH 6.5 – 8.0). This reagent can be purchased from Amresco, Incorporated or VWR International (see List of Recommended Vendors for Phenol:Chloroform:Isoamyl Alcohol in the Hints and Troubleshooting Guide). The phenol:chloroform:isoamyl alcohol may also be user made (please refer to, Preparing Phenol:Chloroform:Isoamyl Alcohol in the Hints and Troubleshooting Guide for preparation instructions). NOTE: Phenol and phenol:chloroform:isoamyl alcohol are subject to oxidation reactions that cause them to become yellow or pink colored, which serves as an indicator that the phenol is NOT useable for RNA extraction. Using colored phenol or colored phenol:chloroform:isoamyl alcohol will result in quality compromised RNA. Prior to each use, a sample of the phenol:chloroform:isoamyl alcohol should be placed in a clear container and its clarity determined. When not in use, store the phenol:chloroform:isoamyl alcohol at 4°C in the dark. Securely cap when not in use and do not expose to light for prolonged periods.

Equipment Required

Centrifuge capable of centrifuging 15 ml tubes (2500 x *g* minimum)
Microcentrifuge (13,000 x *g*)
Pipettor (20 µl to 1000 µl)
Serological pipettes (1 ml and 10 ml)
Heat block (set at 45°C) (optional)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
RNase-Free Gloves (MO BIO Catalog# 1556-S (small), 1556-M (medium) and 1556-L (large))
Lab Cleaner for RNase Removal (MO BIO Catalog# 12095-500)
Vortex Adapter (MO BIO Catalog# 13000-V1-15)

Reagents Required but not Included

Phenol:Chloroform:Isoamyl Alcohol Solution

Kit Contents

	Kit Catalog# 12866-25	
Component	**Catalog #**	**Amount**
Bead Tubes (with 1.5 g beads)	12866-25-PBT	25
Bead Solution	12866-25-BS	69 ml
Solution SR1	12866-25-1	7 ml
Solution SR2	12866-25-2	22 ml
Solution SR3	12866-25-3	42 ml
Solution SR4	12866-25-4	165 ml
Solution SR5*	12866-25-5	110 ml
Solution SR6*	12866-25-6	28 ml
Solution SR7	12866-25-7	3 ml
RNA Capture Columns	12866-25-SF	25
15 ml Collection Tubes	12866-25-T1	100
2.2 ml Collection Tubes	12866-25-T2	25

***Solutions SR5 and SR6 must be shaken before use to ensure consistent results**

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 is flammable. Wear gloves, laboratory coat and safety glasses when handling phenol. Phenol:chloroform:isoamyl alcohol is a caustic organic solution. User should review the vendor provided MSDS and accident procedures for this reagent. Do not inhale vapors. Follow local ordinances for disposal of phenol waste. Phenol is highly corrosive and can cause severe burns. Chloroform is a carcinogen. In the event of an accident, seek medical attention immediately.

IMPORTANT NOTES FOR USE: Phenol:Chloroform:Isoamyl Alcohol

Warning: Phenol and Chloroform are organic compounds that are toxic and potentially dangerous to your health and safety. Please see all manufacturers' warnings and precautions before working with these compounds. Phenol and phenol:chloroform:isoamyl alcohol are subject to oxidation reactions that cause them to become yellow or pink colored, which serves as an indicator that the phenol is NOT useable for RNA extraction. Using colored phenol or colored phenol:chloroform:isoamyl alcohol will result in quality compromised RNA. Prior to each use, a sample of the phenol:chloroform:isoamyl alcohol should be placed in a clear container and its clarity determined. When not in use store the phenol:chloroform:isoamyl alcohol at 4°C in the dark. Securely cap when not in use and do not expose to light for prolonged periods.

Preparing Phenol:Chloroform:Isoamyl Alcohol Solution

Mix 25 parts purified phenol, 24 parts chloroform, and one part isoamyl alcohol. This solution can be stored under TE buffer (10mM Tris, 1mM EDTA, pH 8.0) or 0.1M Tris, pH 8.0, for periods up to 3 months at 4°C. Store in an amber bottle to protect from light. It is recommended, if storing under TE Buffer, to add a small volume of a Tris buffer to this solution.

NOTE: Chloroform is mixed with phenol to increase the efficiency of nucleic acid extractions by reducing losses of DNA and RNA at the phenol:aqueous interphase. Chloroform denatures proteins and aids in the removal of lipids, while isoamyl alcohol reduces foaming during the extraction and facilitates the separation of the aqueous and organic phases.

List of Recommended Vendors for Phenol: Chloroform: Isoamyl Alcohol

Vendor Name	Chemical Name	Catalog Number	Volume (ml)
Amresco, Incorporated*	Phenol: Chloroform (pH 6.7/8.0) 25:24:1 premixed with isoamyl alcohol	0883-100	100
Amresco, Incorporated*	Phenol: Chloroform (pH 6.7/8.0) 25:24:1 premixed with isoamyl alcohol	0883-400	400
VWR International**	Phenol: Chloroform premixed with Isoamyl Alcohol 25:24:1	100513-510	100
VWR International**	Phenol: Chloroform Buffered Solution 25:24:1	IB05174	400

*www.amresco-inc.com or (US) 800.829.2802
** www.vwr.com or (US) 800.932.5000
* **International customers should contact their local distributor

Experienced User Protocol

Wear RNase-Free Gloves (1556) at all times and remove RNase from the work area using Lab Cleaner (12095) for RNase Removal. Both of these products are available from MO BIO. Please see the "Products recommended for you" section at the end of this manual.

1. Add up to 2 g of soil to the 15 ml **Bead Tube** (provided).
   Note: Please refer to Hints and Troubleshooting Guide for information regarding the amount of soil to process.

2. Add 2.5 ml of **Bead Solution** to the Bead Tube followed by 0.25 ml of **Solution SR1** and 0.8 ml of **Solution SR2**.

3. Add 3.5 ml of **phenol:chloroform:isoamyl alcohol** (pH 6.5 – 8.0, [User supplied]) to the bead tube, cap and vortex to mix until the biphasic layer disappears.

4. Place the Bead Tube on the Vortex Adapter (MO BIO Catalog # 13000-V1-15) and vortex at maximum speed for 15 minutes.

5. Remove the Bead Tube from the Vortex Adapter and centrifuge at 2500 x *g* for 10 minutes at room temperature.

6. Remove the Bead Tube from the centrifuge and carefully transfer the upper aqueous phase (avoiding the interphase and lower phenol layer) to a clean **15 ml Collection Tube** (provided). The thickness of the interphase will vary depending on the type of soil used. Discard the phenol: chloroform:isoamyl alcohol in an approved waste receptacle.
   Note: The biphasic layer will be thick and firm in soils high in organic matter and may need to be pierced to remove the bottom phenol layer.

7. Add 1.5 ml of **Solution SR3** to the aqueous phase and vortex to mix. Incubate at 4°C for 10 minutes.

8. Centrifuge at 2500 x g for 10 minutes at room temperature. Transfer the supernatant, without disturbing the pellet (if there is one), to a new **15 ml Collection Tube** (provided).

9. Add 5 ml of **Solution SR4** to the Collection Tube containing the supernatant, invert or vortex to mix, and incubate at room temperature for 30 minutes.
   Note: The previous protocol instructions were to incubate at -20°C for 30 minutes. If you've used the -20°C incubation before and know that your soil type yields good results at that temperature, you may continue to follow that protocol.

10. Centrifuge at 2500 x *g* for 30 minutes at room temperature.

11. Decant the supernatant and invert the 15 ml Collection Tube on a paper towel for 5 minutes.
    Note: Depending on soil type, the pellet may be large and/or dark in color.

12. Shake Solution SR5 to mix. Add 1 ml of **Solution SR5** to the 15 ml Collection Tube and resuspend the pellet completely by repeatedly pipetting or vortexing to disperse the pellet.

Note: Depending on the soil type, the pellet may be difficult to resuspend. Resuspension may be aided by placing the tubes in a heat block or water bath at 45°C for 10 minutes, followed by vortexing. Repeat until the pellet is resuspended.

13. Prepare one **RNA Capture Column** (provided) for each RNA Isolation Sample:
    a. Remove the cap of a **15 ml Collection Tube** (provided) and place the **RNA Capture Column** inside the **15 ml Collection Tube**. The column will hang in the **15 ml Collection Tube**.

    b. Add 2 ml of **Solution SR5** to the **RNA Capture Column** and allow it to gravity flow through the column and collect in the 15 ml Collection Tube. Allow **Solution SR5** to completely flow through the column (**Optional**: The Collection Tube may be emptied after Solution SR5 has completely flowed through the column. **Note**: DO NOT ALLOW THE COLUMN TO DRY OUT PRIOR TO LOADING THE RNA ISOLATION SAMPLE.)

14. Add the RNA Isolation Sample from Step 12 onto the **RNA Capture Column** and allow it to gravity flow through the column. Collect the flow through in the 15 ml Collection Tube.

15. Wash the column with 1 ml of **Solution SR5**. Allow it to gravity flow and collect the flow through in the 15 ml Collection Tube.

16. Transfer the **RNA Capture Column** to a new **15 ml Collection Tube** (provided). Shake Solution SR6 to mix and then add 1 ml of **Solution SR6** to the **RNA Capture Column** to elute the bound RNA into the **15 ml Collection Tube**. Allow **Solution SR6** to gravity flow into the **15 ml Collection Tube**.
    Note: The RNA PowerSoil® DNA Elution Accessory Kit is available for DNA elution (MO BIO Catalog# 12867-25). See the DNA Elution Procedure in the Hints and Troubleshooting Guide or contact MO BIO for details at technical@mobio.com.

17. Transfer the eluted RNA to a **2.2 ml Collection Tube** (provided) and add 1 ml of **Solution SR4**. Invert at least once to mix and incubate at -20°C for a minimum of 10 minutes.

18. Centrifuge the **2.2 ml Collection Tube** at 13,000 x *g* for 15 minutes at room temperature to pellet the RNA.

19. Decant the supernatant and invert the 2.2 ml Collection Tube onto a paper towel for 10 minutes to air dry the pellet.

20. Resuspend the RNA pellet in 100 µl of **Solution SR7**. For information on removal of genomic DNA from RNA, see the Hints and Troubleshooting Guide on page 13.

Thank you for choosing the RNA PowerSoil® Total RNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)

Wear RNase-Free Gloves (1556) at all times and remove RNase from the work area using Lab Cleaner (12095) for RNase Removal. Both of these products are available from MO BIO. Please see the "Products recommended for you" section at the end of this manual.

1. Add up to 2 g of soil to the 15 ml **Bead Tube** (provided).
   Note: Please refer to Hints and Troubleshooting Guide for information regarding the amount of soil to process.

2. Add 2.5 ml of **Bead Solution** to the Bead Tube followed by 0.25 ml of **Solution SR1** and 0.8 ml of **Solution SR2**.

   What's happening: The Bead Solution is a buffer used to disperse cells and soil particles. Solution SR1 contains SDS and other disruption agents which aid in complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. Solution SR2 is a precipitation reagent used to remove non-DNA organic and inorganic material including cell debris and proteins. ***Note:*** *If it gets cold, Solution SR1 will form a white precipitate. Heating to 60ºC will dissolve the SDS and will not harm the other disruption agents.*

3. Add 3.5 ml of phenol:chloroform:isoamyl alcohol (pH 6.5 – 8.0, [User supplied]) to the bead tube, cap and vortex to mix until the biphasic layer disappears.

   What's happening: Phenol:chloroform:isoamyl alcohol is added to maximize lysing efficiency and yield. Lysed cell components are trapped in the solvent and proteins are denatured leaving the nucleic acid in solution.

4. Place the Bead Tube on the Vortex Adapter (MO BIO Catalog# 13000-V1-15) and vortex at maximum speed for 15 minutes.

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   What's happening: Cells are lysed by combination of chemical agents from steps 1-3 and the mechanical shaking introduced by vortexing. The MO BIO Vortex Adapter is designed to be a simple cost effective platform to facilitate the homogenization and cell lysis of samples. An alternative to the MO BIO Vortex Adapter would be to attach your tubes to your platform with tape. Note that tape can become loose and may result in uneven shaking and lysis efficiency resulting in inconsistent results or lower yields.

5. Remove the Bead Tube from the Vortex Adapter and centrifuge at 2500 x *g* for 10 minutes at room temperature.

   What's happening: Centrifugation results in phase separation of the sample mixture. Three phases will be visible after centrifugation. The lower organic phase containing proteins and cellular debris, the interphase containing humics and other organic and non-organic material, and the upper aqueous phase containing total nucleic acid. ***Note:*** *The thickness of the interphase will depend on the sample type. Samples high in organic content will have a thicker interphase.*

6. Remove the Bead Tube from the centrifuge and carefully transfer the upper aqueous phase (avoiding the interphase and lower phenol layer) to a clean **15 ml Collection Tube** (provided). The thickness of the interphase will vary depending on the type of soil used.
   Note: Discard the phenol:chloroform:isoamyl alcohol in an approved waste receptacle. The biphasic layer will be thick and firm in soils high in organic matter and may need to be pierced to remove the bottom phenol layer for disposal.

What's happening: The upper aqueous phase containing the total nucleic acids from the sample is transferred to a new tube. Cellular debris, proteins, and organic material are left behind. Take care not to transfer material from the lower phase or the interphase.

7. Add 1.5 ml of **Solution SR3** to the aqueous phase and vortex to mix. Incubate at 4°C for 10 minutes.

   What's happening: Solution SR3 is a secondary precipitation step to further remove proteins and cellular debris.

8. Centrifuge at 2500 x g for 10 minutes at room temperature. Transfer the supernatant without disturbing the pellet (if there is one) to a new **15 ml Collection Tube** (provided).

   What's happening: The supernatant containing nucleic acids are transferred to a new 15 ml tube. Non-nucleic acid material is left behind.

9. Add 5 ml of **Solution SR4** to the Collection Tube containing the supernatant, invert or vortex to mix, and incubate at room temperature for 30 minutes.
   Note: The previous protocol instructions were to incubate at -20°C for 30 minutes. If you've used the -20°C incubation before and know that your soil type yields good results at this temperature, you may continue to follow that protocol.

10. Centrifuge at 2500 x *g* for 30 minutes at room temperature.

11. Decant the supernatant and invert the 15 ml Collection Tube on a paper towel for 5 minutes.
    Note: Depending on soil type, the pellet may be large and/or dark in color.

    What's happening: Solution SR4 is 100% Isopropanol. Nucleic acid is precipitated and the Isopropanol is discarded.

12. Shake Solution SR5 to mix. Add 1 ml of **Solution SR5** to the **15 ml Collection Tube** and resuspend the pellet completely by repeatedly pipetting or vortexing to disperse the pellet.
    Note: Depending on the soil type, the pellet may be difficult to resuspend. Resuspension may be aided by placing the tubes in a heat block or water bath at 45°C for 10 minutes, followed by vortexing. Repeat until the pellet is resuspended.

    What's happening: Solution SR5 is a proprietary salt solution used to resuspend the precipitated nucleic acids from step 11. It is also used to equilibrate the RNA capture column in step 13 and to wash and prep the column for the elution of RNA in step 17 below.

13. Prepare one **RNA Capture Column** (provided) for each RNA Isolation Sample:
    a. Remove the cap of a **15 ml Collection Tube** (provided) and place the **RNA Capture Column** inside the **15 ml Collection Tube**. The column will hang in the **15 ml Collection Tube**.
    b. Add 2 ml of **Solution SR5** to the **RNA Capture Column** and allow it to gravity flow through the column and collect in the **15 ml Collection Tube**. Allow **Solution SR5** to completely flow through the column (**Optional**: The Collection Tube may be emptied after Solution SR5 has completely flowed through the column. **Note**: DO NOT ALLOW THE COLUMN TO DRY OUT PRIOR TO LOADING THE RNA ISOLATION SAMPLE.)

14. Add the RNA Isolation Sample from Step 12 onto the **RNA Capture Column** and allow it to gravity flow through the column. Collect the flow through in the 15 ml Collection Tube.

15. Wash the column with 1 ml of **Solution SR5**. Allow it to gravity flow and collect the flow through in the 15 ml Collection Tube.

    What's happening: The sample is added to the RNA Capture Column and the nucleic acids are bound to the column matrix. The Capture Column is then washed with a second volume of Solution SR5 to ensure unbound contaminants are removed from the sample and column prior to the elution of RNA.

16. Transfer the **RNA Capture Column** to a new **15 ml Collection Tube** (provided). Shake Solution SR6 and then add 1 ml of **Solution SR6** to the **RNA Capture Column** to elute the bound RNA into the **15 ml Collection Tube**. Allow **Solution SR6** to gravity flow into the **15 ml Collection Tube**.

    What's happening: The Solution SR6 RNA elution buffer is a proprietary salt solution that allows for the preferential release of RNA from the RNA Capture Column leaving DNA, residual debris, and inhibiting substances in the column.

    Note: The RNA PowerSoil® DNA Elution Accessory Kit is available for DNA elution (MO BIO Catalog# 12867-25). See the DNA Elution Procedure in the Hints and Troubleshooting Guide or contact MO BIO for details at technical@mobio.com.

17. Transfer the eluted RNA to a **2.2 ml Collection Tube** (provided) and add 1 ml of **Solution SR4**. Invert at least once to mix and incubate at -20°C for a minimum of 10 minutes.

18. Centrifuge the **2.2 ml Collection Tube** at 13,000 x *g* for 15 minutes at room temperature to pellet the RNA.

19. Decant the supernatant and invert the 2.2 ml Collection Tube onto a paper towel for 10 minutes to air dry the pellet.

    What's happening: Solution SR4 is 100% Isopropanol. Eluted RNA from the Capture Column is precipitated, centrifuged, and allowed to air dry prior to resuspending and concentrating.

20. Resuspend the RNA pellet in 100 µl of **Solution SR7**. For information on removal of genomic DNA from RNA, see the Hints and Troubleshooting Guide on page 13.

    What's happening: Solution SR7 is RNase/DNase-Free water used to resuspend the pelleted RNA. Solution SR7 contains no EDTA. RNA is now ready for any downstream application. For long term storage of samples 10 mM Tris pH 8.0 or TE buffer may be used to resuspend the pelleted RNA.

Thank you for choosing the RNA PowerSoil® Total RNA Isolation Kit.

Protocol for LifeGuard™ Soil Preservation Solution

1. Add between 2 and 2.5 volumes of LifeGuard™ Soil Preservation Solution per gram of soil. For example 1 gram of soil would require 2 to 2.5 ml of LifeGuard™. If you are collecting the soil directly into the RNA PowerSoil® 15 ml Bead Tube, add 5 ml of LifeGuard™ into a 2 gram soil sample.

   Note: If you are working with sediment samples, use 3 volumes of LifeGuard™ per gram of wet weight.

2. Vortex or hand-mix the solution and soil until the entire sample is saturated with solution. Excess LifeGuard™ Solution should be sitting on top of your soil sample.

3. Store at -20°C, 4°C, or room temperature as desired. When ready to process the RNA, centrifuge the sample at 2500 x *g* for 5 minutes to collect the soil. Remove the LifeGuard™ Soil Preservation Solution from the tube.

4. If you collected soil directly in the 15 ml Bead Tube, you can remove the LifeGuard™ after centrifugation and continue at step 2 of the RNA PowerSoil® Total RNA Isolation Kit protocol.

5. If you stabilized soil in another vessel, weigh the amount of soil you need and transfer to the 15 ml Bead Tube, or a conical tube, and then centrifuge to remove the LifeGuard™ Solution.

A number of different soils have been tested using LifeGuard™ and each soil will differ in its biomass and its saturation with water. Sediment samples require a higher volume of LifeGuard™ (3:1) due to dilution of the reagent.

Biomass content may play a role in the stabilization of soils at different temperatures and the ability of LifeGuard™ to freeze the community profile. For a soil with unknown microbial content or where temperatures may exceed room temperature (22-25^{o}C), it may be preferable to store the soil at -20^{o}C or 4^{o}C to ensure the original community profile is maintained and to use more than 2.5 ml of LifeGuard™ per gram of soil. For long term storage and transport (>30 days), store stabilized soil at -20^{o}C.

Hints and Troubleshooting Guide

Soil Types and Soil Amount to Process

The yield and purity of RNA will depend on the soil type processed. The RNA PowerSoil® Total RNA Isolation Kit has been validated with diverse soil types that represent a wide range of physical, chemical and biological characteristics. In our experience, it is possible to use up to a maximum of 2 g for most soil types. For soils with high organic content, 1 g of soil typically gives an adequate amount of RNA while reducing the potential for DNA carryover during purification.

For sediment samples, more soil may be used. We have used up to 5 grams of sediment with the RNA PowerSoil® Total RNA Isolation Kit. You may increase the volume of Solution SR4 at step 9 to equal the volume of lysate.

Some soils and sediments may contain excess salinity. This is visualized after step 10 by a large white or yellow salty pellet. This type of pellet may result in reduced nucleic acid yields. If you have a salt enriched soil or sediment, to overcome the precipitation of salt in the pellet after Solution SR4, incubate the samples at room temperature for 30 minutes instead of -20°C. Proceed as directed for the rest of the protocol.

Phase Separation of Phenol:Chloroform:Isoamyl Alcohol

To ensure effective phenol:chloroform:isoamyl alcohol and aqueous phase separation, centrifuge 15 ml tubes at 2,500 x *g* at room temperature for at least 10 minutes (step 5). Following centrifugation, the interphase thickness between the phenol:chloroform:isoamyl alcohol and aqueous layers will vary depending on the organic content of the soil. Care should be taken to avoid the lower phenol phase and the interphase containing protein and lipid when removing the aqueous upper phase. If a portion of the phenol layer or the interphase is removed, recentrifuge the transferred aqueous phase containing tube to obtain a phase separation that will permit removing the aqueous phase. Alternatively, an equal volume (2 ml) of chloroform may be added to the phenol or interphase contaminated aqueous phase, inverted or vortexed to mix, and the tubes centrifuged at 2,500 *x g* for 10 minutes at room temperature. Remove the upper aqueous phase and discard the lower chloroform:phenol interphase. Then combine with the rest of the aqueous phase and continue with protocol.

Pellet Resuspension in Solution SR5

Soil types with a high organic content may yield RNA pellets that are difficult to resuspend. Heating the RNA pellet in Solution SR5 at 45°C will aid in the resuspension process. Disrupting the RNA pellet with a pipette tip and vortexing vigorously will also aid in RNA resuspension. It is important to resuspend the pellet completely before applying it to the column in step 14. Failure to completely resupend the RNA pellet will result in RNA loss through reduced column binding and will result in reduced column flow rate.

Column Flow

The RNA Capture Columns are rated for gravity flow and should not be used with centrifugal or vacuum force. Additional positive pressure can be applied to the column using a 5 cc syringe barrel. Hold the 5 cc syringe barrel flush against the top of the RNA Capture Column and gently push the syringe inside the 5 cc barrel to force air through the RNA Capture Column. Flow rate should not exceed 1 drop per second.

Hints and Troubleshooting Guide cont.

DNA Contamination of the RNA

DNA carryover typically does not occur with the majority of soil types, however certain soils high in organic matter may present unique carryover situations. The purified RNA may be tested for potential DNA carryover by agarose gel analysis or by performing PCR with qualified primers on the isolated RNA without performing prior reverse transcription amplification. The absence of a detectable amplification fragment by agarose electrophoresis indicates the absence of detectable carryover DNA. In the event DNA is detected, DNase treatment of the isolated RNA is recommended.

For removal of genomic DNA from RNA preps, we recommend the RTS DNase Kit (MO BIO Catalog# 15200-50). The RTS DNase Kit uses a high velocity DNase enzyme which is efficiently removed post-digestion using a resin which inactivates and binds to the enzyme. RNA is purified away from DNase without the use of inhibitors (EDTA) or heat and is ready to use in your next application.

Multiple Elutions from the Same Column

Multiple elutions beyond those called for in the protocol are not recommended when using the Capture Columns. Although a small amount of additional RNA or DNA (if using the RNA PowerSoil® DNA Elution Accessory Kit, MO BIO Catalog# 12867-25) may come off the column with multiple elutions, the inhibitors associated with the starting material will also begin to wash off the column and could cause inhibition in downstream applications.

Co-Isolation of DNA from the RNA Capture Column

After the RNA is eluted from the RNA capture column the DNA may also be eluted from the same column using the RNA PowerSoil® DNA Elution Accessory Kit (MO BIO Catalog# 12867-25). The capture column is placed in a new 15 ml tube and the DNA elution buffer is added to the column and the DNA is eluted in a separate tube. Everything required for DNA isolation is provided in the kit.

DNA Elution Procedure
(RNA PowerSoil® DNA Elution Accessory Kit Catalog #12867-25 Required)

1. Transfer the RNA Capture Column from step 19 of the RNA PowerSoil® Total RNA Isolation Kit (MO BIO Catalog #12866-25) to a 15 ml Collection Tube (provided). Shake **Solution SR8**, then add 1 ml of **Solution SR8** to the Capture Column to elute the bound DNA into the 15 ml Collection Tube. Allow Solution SR8 to gravity flow through the 15 ml Collection Tube.

2. Transfer the eluted DNA to a **2.2 ml Collection Tube** (provided) and add 1 ml of **Solution SR4**. Invert at least once to mix and incubate at -20°C for a minimum of 10 minutes.

3. Centrifuge the 2.2 ml Collection Tube at 13,000 x *g* for 15 minutes at room temperature to pellet the DNA.

4. Decant the supernatant and invert the 2.2 ml Collection Tube onto a paper towel for 10 minutes to air dry the pellet.

5. Resuspend the DNA pellet in 100 µl of Solution SR7.

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
RNA PowerSoil® DNA Elution Accessory Kit	12867-25	25 preps
LifeGuard™ Soil Preservation Solution	12868-100 12868-1000	100 ml 1000 ml
RTS DNase™ Kit	15200-50	50 preps
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
PowerLyzer® PowerSoil® DNA Isolation Kit	12855-50 12855-100	50 preps 100 preps
PowerMicrobiome™ RNA Isolation Kit	26000-50	50 preps
PowerWater® RNA Isolation Kit	14700-50-NF	50 preps
PowerPlant® RNA Isolation Kit	13500-50	50 preps
UltraClean® Lab Cleaner	12095-500 12095-1000	500 ml squeeze bottle 1 L bottle
RNase-Free Gloves	1556-XS 1556-S 1556-M 1556-L	X-Small (Bag of 150) Small (Bag of 150) Medium (Bag of 150) Large (Bag of 150)