# RNA PowerSoil®
# DNA Elution Accessory Kit

| Catalog No. | Quantity |
|---|---|
| 12867-25 | 25 Preps |

***Instruction Manual***

**Please Recycle**

**Version:** 04282014

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## Introduction

MO BIO Laboratories' RNA PowerSoil® DNA Elution Accessory Kit is designed to co-isolate DNA for the recovery of the total nucleic acid content of the original sample. When used in combination with the RNA PowerSoil® Total RNA Isolation Kit (catalog #12866-25) both the RNA and the DNA can be isolated and eluted into two separate fractions.

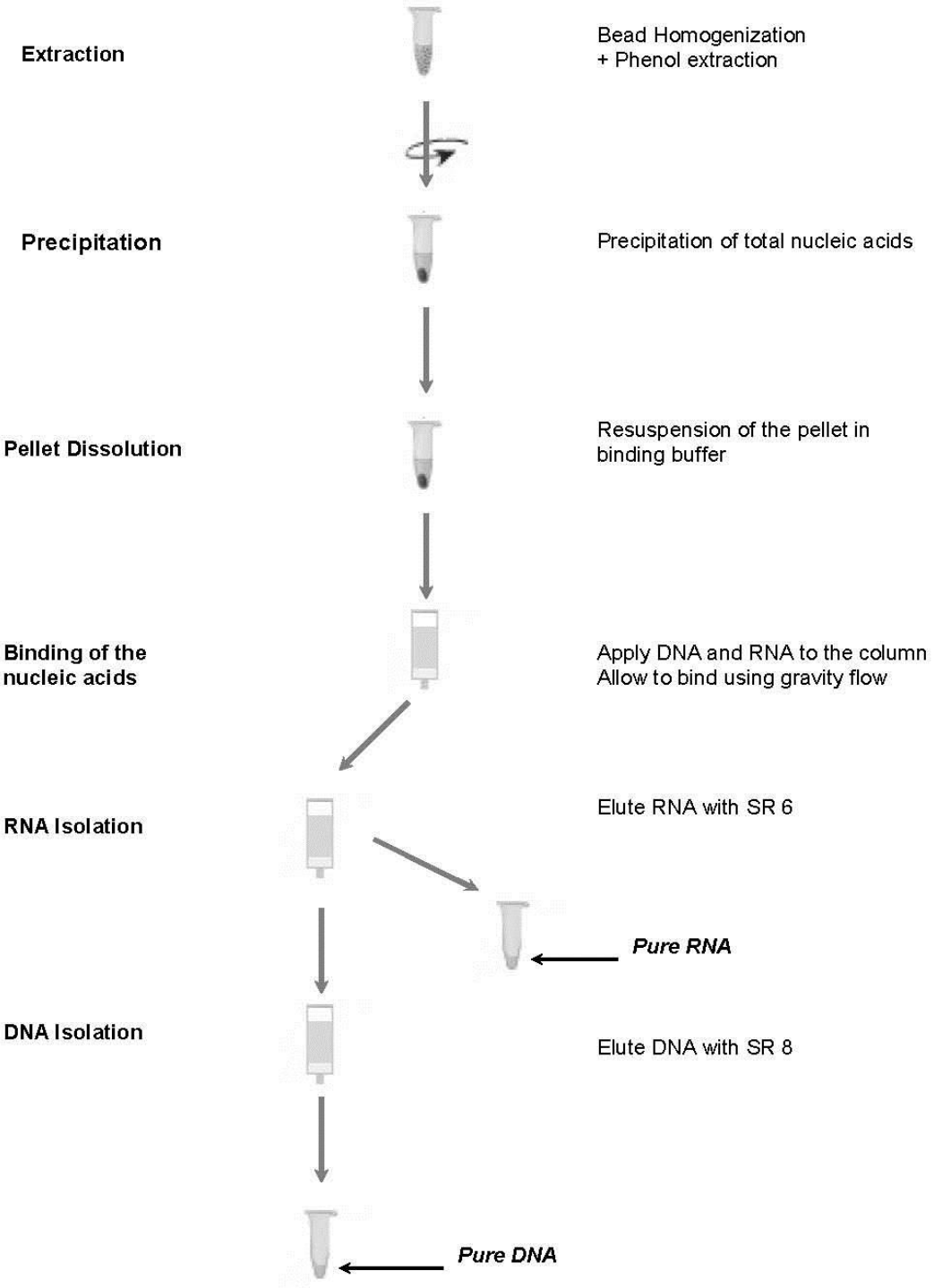
## Protocol Overview

After the RNA is eluted from the RNA capture column using the RNA PowerSoil® Total RNA Isolation Kit, the column is placed in a new 15 ml tube. The DNA elution buffer is added to the column and DNA is preferentially eluted from the proprietary matrix of the capture column. DNA is then concentrated and ready for use for downstream applications without further handling or purification.

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

| Other Related Products | Catalog No. | Quantity |
|---|---|---|
| RNA PowerSoil® Total RNA Isolation Kit | 12866-25 | 25 preps |
| RNase-Free Gloves | 1556-XS<br>1556-S<br>1556-M<br>1556-L | bag of 150<br>bag of 150<br>bag of 150<br>bag of 150 |
| UltraClean® Lab Cleaner | 12095-500<br>12095-1000 | 500 ml spray bottle<br>1 liter bottle |

## RNA PowerSoil® DNA Elution Accessory Kit



**Equipment Required:**
Microcentrifuge (13,000 x *g*)
Pipettor (20 µl to 1000 µl)
Serological pipettes (1 ml and 10 ml)
Vortex
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, 12095-1000)
Vortex Adapter (MO BIO Catalog # 13000-V1-15)

**Reagents Required but Not Included**
RNA PowerSoil® RNA Isolation Kit (MO BIO Catalog # 12866-25)

**Kit Contents**

| | **Kit Catalog# 12867-25** | |
|---|---|---|
| **Component** | **Catalog #** | **Amount** |
| Solution SR4 | 12867-25-1 | 27.5 ml |
| Solution SR7 | 12867-25-2 | 3 ml |
| Solution SR8 | 12867-25-3 | 27.5 ml |
| 15 ml Collection Tubes | 12867-25-T1 | 25 |
| 2.2 ml Collection Tubes | 12867-25-T2 | 25 |

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

**Precautions**
Please wear gloves, laboratory coat and safety glasses when using this product. Avoid skin contact with kit reagents. In case of contact, wash the affected area thoroughly with soap and water. Do not ingest. See Material Safety Data Sheets (MSDS) for emergency procedures in case of accidental contact or ingestion. MSDS information is available upon request (760-929-9911) or at www.mobio.com.

**WARNING:** Reagent SR4 is flammable and should be kept away from open flames and sparks.

**IMPORTANT NOTE FOR USE:** Wear RNase and DNase-Free gloves at all times. Remove RNases and DNases from work surfaces with Lab Cleaner before starting. These products are available separately from MO BIO Laboratories, Inc. (refer to the "Equipment Required" section).

# Experienced User Protocol

**Please wear gloves at all times.**

## DNA Elution Procedure

1. Transfer the RNA Capture Column from step 16 of the RNA PowerSoil® Total RNA Isolation Kit (MO BIO Catalog # 12866-25) to a **15 ml Collection Tube** (provided) and add 1 ml of **Solution SR8** to the RNA Capture Column to elute the bound DNA into the **15 ml Collection Tube**. Allow **Solution SR8** to gravity flow into 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 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**.
   **Note:** Although RNA carryover does not occur with the majority of soil types, certain soils high in organic matter may present unique carryover situations. In situations where the absence of RNA contamination is critical, an RNase treatment of the isolated DNA is recommended; see the Hints and Troubleshooting Guide for instruction.

**Thank you for choosing the RNA PowerSoil® DNA Elution Accessory Kit.**

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

## DNA Elution Procedure

1. Transfer the RNA Capture Column from step 16 of the RNA PowerSoil® Total RNA Isolation Kit (catalog #12866-25) to a **15 ml Collection Tube** (provided) and add 1 ml of **Solution SR8** to the RNA Capture Column to elute the bound DNA into the **15 ml Collection Tube**. Allow **Solution SR8** to gravity flow into the **15 ml Collection Tube**.

   *What's happening: The Solution SR8 elution buffer is a salt solution that allows for the preferential release of DNA from the RNA Capture Column leaving residual debris and inhibiting substances in the column.*

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 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.

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

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

   *What's happening: Solution SR7 is RNase/DNase-Free water used to resuspend the pelleted DNA. Solution SR7 contains no EDTA. DNA 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 DNA.*

   **Note:** Although RNA carryover does not occur with the majority of soil types, certain soils high in organic matter may present unique carryover situations. In situations where the absence of RNA contamination is critical, an RNase treatment of the isolated DNA is recommended; see Additional Information Section for instruction).

**Thank you for choosing the RNA PowerSoil® DNA Elution Accessory Kit.**

# Hints and Troubleshooting Guide

### *Soil Types and Soil Amount Processed*

The yield and purity of DNA and RNA isolated using the RNA PowerSoil® Total RNA Isolation Kit in combination with the RNA PowerSoil® DNA Elution Accessory Kit will depend on the soil type processed. A wide range of soil types can be processed with different physical, chemical and biological characteristics including compost, manure, estuary sediment, and other soil types high in organic content. 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, as little as 0.25 g of soil will yield an adequate amount of total nucleic acid from a sample and reduce the potential for DNA and RNA elution crossover between fractions. We recommend at most 0.25-0.5 g for absorbent soils like potting soil or peat.

### *Column Flow*

The RNA Capture Columns used in the RNA PowerSoil® Total RNA Isolation Kit and in combination with the RNA PowerSoil® DNA Elution Accessory Kit are rated for gravity flow and should not be used with centrifugal or vacuum force.

### *DNase/RNase Digestion Procedure*

**Note**: ONLY RNase-Free DNase may be used with this protocol. The presence of RNases will result in digested RNA.

The presence of carryover DNA with RNA or vice versa isolated using the RNA PowerSoil® Total RNA Isolation Kit in combination with the RNA PowerSoil® DNA Elution Accessory Kit does not occur with the majority of soil types. It has been noted, however, that soils with high organic matter content may show crossover of DNA or RNA between fractions.

The following protocol should serve as a guide to the enzymatic digestion of either the DNA or RNA in the fraction of interest.

a. Add the appropriate amount of enzyme buffer and or water and up to 4 Units of DNase or RNase enzyme to the nucleic acid sample to obtain a total volume of 200 µl. A typical 10X DNase/RNase digestion buffer is 10 mM $CaCl_2$ and 10 mM $MgCl_2$ in 10 mM Tris-HCl buffer, pH 7.5.
b. Incubate at 37°C for 30 to 45 minutes.
c. Add 200 µl of phenol:chloroform:isoamyl alcohol (pH 6.5 – 8.0) and vortex to mix. Incubate at room temperature for 5 minutes.
d. Centrifuge the sample at 10,000 *x g* for 5 minutes.
e. Carefully remove the upper aqueous phase and transfer it to a new tube.
f. Add 1/10$^{th}$ volume of 5M NaCl, two volumes of 100% ethanol and invert to mix.
g. Incubate at -20°C for 30 minutes and centrifuge at 10,000 *x g* for 10 minutes.
h. Decant the supernatant and air dry the pellet.
i. Resuspend the pellet in an appropriate volume of Solution SR7.

### *Multiple Elutions from the Same Column*

Multiple elutions beyond those called for in the protocol are not recommended when using the RNA Capture Columns. Although a small amount of additional RNA or DNA 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.

## Contact Information

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

**Ordering Information:**
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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](http://www.mobio.com)*

| Description | Catalog No. | Quantity |
|---|---|---|
| RNA PowerSoil® Total RNA Isolation Kit | 12866-25 | 25 preps |
| LifeGuard™ Soil Preservation Solution | 12868-100<br>12868-1000 | 100 ml<br>1000 ml |
| PowerSoil® DNA Isolation Kit | 12888-50<br>12888-100 | 50 preps<br>100 preps |
| Vortex-Genie® 2 Vortex | 13111-V<br>13111-V-220 | 1 unit (120V)<br>1 unit (220V) |
| Vortex Adapter for Vortex Genie® 2 | 13000-V1-15 | Holds 4 (15 ml) Tubes |
| RTS DNase™ Kit | 15200-50 | 50 preps |