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RNeasy® PowerSoil® DNA Elution Kit Handbook

For the co-isolation of DNA and RNA from soil using the RNeasy PowerSoil Total RNA Kit

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy PowerSoil DNA Elution Kit	**(25)**
Catalog no.	**12867-25**
Number of preps	**25**
Solution SR4	27 ml
Solution SR7	2 x 1.5 ml
Solution SR8	27.5 ml
Collection Tubes (15 ml)	50
Collection Tubes (2.2 ml)	25
Quick Start Protocol	1

Storage

The RNeasy PowerSoil DNA Elution Kit reagents and components can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

All RNeasy products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Warning: Solution SR4 is flammable and should be kept away from open flames and sparks.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of RNeasy PowerSoil DNA Elution Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAGEN RNeasy PowerSoil DNA Elution 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 RNeasy PowerSoil Total RNA Kit (cat. no. 12866-25), both RNA and DNA can be isolated and eluted into two separate fractions.

Principle and procedure

After RNA is eluted from the RNA Capture Column using the RNeasy PowerSoil Total RNA Kit, the column is placed in a new 15 ml tube. 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 in downstream applications.

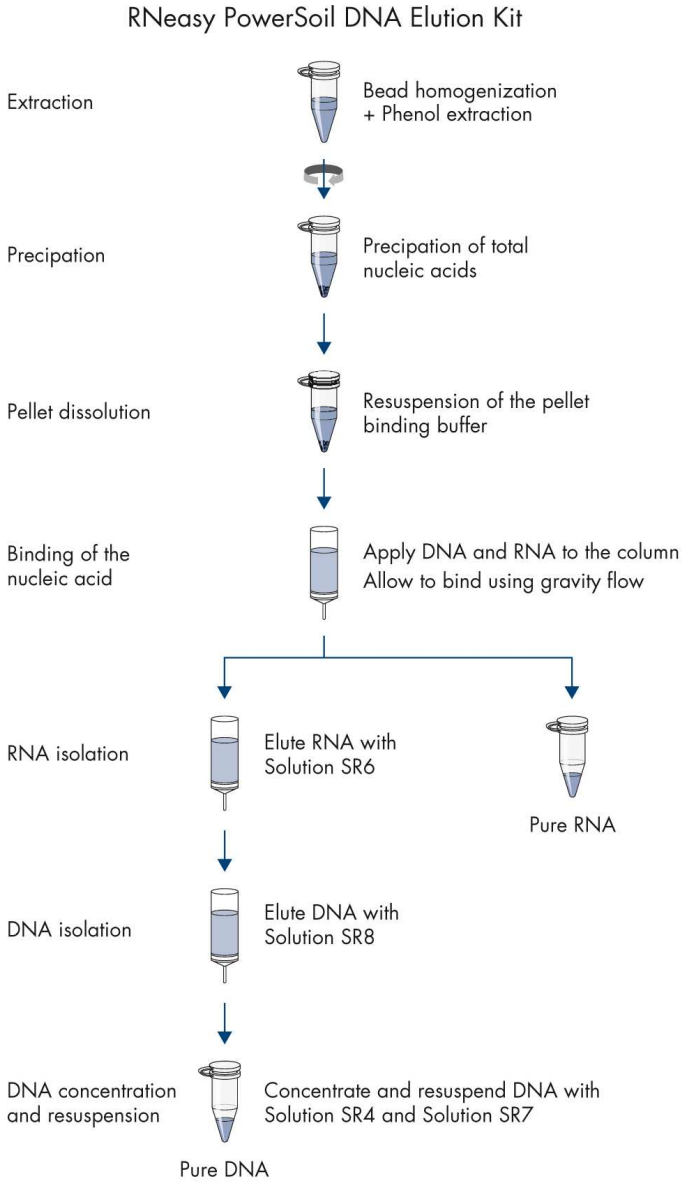

Figure 1. RNeasy PowerSoil DNA Elution Kit procedure.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

- Microcentrifuge (13,000 x *g*)
- Serological pipettes (1 ml and 10 ml)
- Pipettor (20–1000 µl)
- RNase-free gloves (cat. nos. 1556-S, 1556-M and 1556-L)
- Lab cleaner for RNase removal (cat. no. 12095-500)
- RNeasy PowerSoil Total RNA Kit (cat. no. 12866-25)

Protocol: Experienced User

Notes before starting

- Wear RNase- and DNase-free gloves at all times.
- Remove RNases and DNases from work surfaces before starting.

Procedure

1. Transfer the RNA Capture Column from step 16 of the RNeasy PowerSoil Total RNA Kit (cat. no. 12866-25) to a 15 ml Collection Tube (provided).
2. 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 Collection Tube.
3. 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 –15°C to –30°C for 10 min.
4. Centrifuge the 2.2 ml Collection Tube at 13,000 x *g* for 15 min at room temperature to pellet the DNA.
5. Decant the supernatant and invert the 2.2 ml Collection Tube onto a paper towel for 10 min to air dry the DNA pellet.
6. Resuspend the DNA pellet in 100 µl of Solution SR7.
   Note: Although RNA carryover does not occur with most soil types, certain soils high in organic matter may present unique carryover situations. When the absence of RNA contamination is critical, an RNase treatment of the isolated DNA is recommended; please refer to the Troubleshooting Guide for instructions.

Protocol: Detailed

Notes before starting

- Wear RNase- and DNase-free gloves at all times.
- Remove RNases and DNases from work surfaces before starting.

Procedure

1. Transfer the RNA Capture Column from step 16 of the RNeasy PowerSoil Total RNA Kit (cat. no. 12866-25) to a 15 ml Collection Tube (provided).
2. 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 Collection Tube.
   Note: Solution SR8 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.
3. 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 –15°C to –30°C for 10 min.
4. Centrifuge the 2.2 ml Collection Tube at 13,000 x *g* for 15 min at room temperature to pellet the DNA.
5. Decant the supernatant and invert the 2.2 ml Collection Tube onto a paper towel for 10 min to air dry the DNA pellet.
   Note: Solution SR4 is 100% Isopropanol. DNA eluted from the RNA Capture Column is precipitated, centrifuged and allowed to air dry before resuspending and concentrating.
6. Resuspend the DNA pellet in 100 µl of Solution SR7.
   Note: Solution SR7 is RNase/DNase-free water and is used to resuspend the pelleted DNA. Solution SR7 contains no EDTA. The eluted DNA is now ready for downstream applications. 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 most soil types, certain soils high in organic matter may present unique carryover situations. When the absence of RNA contamination is critical, an RNase treatment of the isolated DNA is recommended; please refer to the Troubleshooting Guide for instructions.

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Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies. For contact information, visit **www.qiagen.com**.

		Comments and suggestions
Soil processing		
a)	Processing different amounts and types of soil	The yield and purity of DNA and RNA isolated using the RNeasy PowerSoil Total RNA Kit in combination with the RNeasy PowerSoil DNA Elution Kit will depend on the type of soil being processed. A wide range of soil types with different physical, chemical and biological characteristics including compost, manure, estuary sediment, and other soil types high in organic content can be processed using these kits. 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 and reduce the potential for DNA and RNA elution crossover between fractions. We recommend starting with at most 0.25–0.5 g for absorbent soils like potting soil or peat.
Procedure		
a)	Column flow	The RNA Capture Columns are rated for gravity flow and should not be used with centrifugal or vacuum force.
b)	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 disrupt downstream applications.

		Comments and suggestions
c)	DNase/RNase digestion protocol	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 RNeasy PowerSoil Total RNA Kit in combination with the RNeasy PowerSoil DNA Elution Kit does not occur with the majority of soil types. However, 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 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 min. c) Add 200 µl of phenol/chloroform/isoamyl alcohol (pH 6.5–8.0) and vortex to mix. Incubate at room temperature for 5 min. d) Centrifuge the sample at 10,000 x *g* for 5 min. e) Carefully remove the upper aqueous phase and transfer it to a new tube. f) Add 1/10th volume of 5M NaCl, two volumes of 100% ethanol and invert to mix. g) Incubate at –20°C for 30 min and centrifuge at 10,000 x *g* for 10 min. h) Decant the supernatant and air dry the pellet. i) Resuspend the pellet in an appropriate volume of Solution SR7.

Ordering Information

Product	Contents	Cat. no.
RNeasy PowerSoil DNA Elution Kit (25)	For 25 preps: Co-isolate DNA and RNA from soil using the RNeasy PowerSoil Total RNA Kit	12867-25
RNeasy PowerSoil Total RNA Kit (25)	For 25 preps: Isolate high-quality total RNA from all soil types	12866-25
LifeGuard Soil Preservation (100 ml)	Stabilize ambient temperature of microbial RNA in soil	12868-100
LifeGuard Soil Preservation (1000 ml)	Stabilize ambient temperature of microbial RNA in soil	12868-1000
DNeasy PowerSoil Kit (50)	For 50 preps: Isolate microbial genomic DNA from all soil types	12888-50
DNeasy PowerSoil Kit (100)	For 100 preps: Isolate microbial genomic DNA from all soil types	12888-100
DNase Max® Kit (50)	For 50 preps: Removal of genomic DNA contamination in RNA preparations using a high activity DNase I enzyme and a highly specific DNase removal resin	15200-50

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Limited License Agreement for RNeasy PowerSoil DNA Elution Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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