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RNeasy® PowerClean® Pro Cleanup Kit Handbook

For the removal of PCR inhibitors from purified RNA in just 7 minutes

Sample to Insight

QIAGEN

Contents

Kit Contents

RNeasy PowerClean Pro Cleanup Kit	**(50)**
Catalog no.	**13997-50**
Number of preps	**50**
MB RNA Spin Columns	50
Solution CU	4 ml
Solution IR	2 x 1.5 ml
Solution SB	22 ml
Solution RW	2 x 28 ml
RNase-Free Water	2 x 10 ml
Collection Tubes (2 ml)	3 x 50
Quick Start Protocol	1

Storage

The RNeasy PowerClean Pro Cleanup Kit can be stored at room temperature (15–25°C) until the expiration date printed on the 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 RW contains ethanol and is flammable.**

CAUTION 	**DO NOT add bleach or acidic solutions directly to the sample preparation waste**

Solution CU and Solution SB contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Introduction

The RNeasy PowerClean Pro Cleanup Kit uses Inhibitor Removal Technology® (IRT) to provide researchers with a novel and proprietary method for cleaning up previously isolated RNA.

Starting RNA may be amber to brown in appearance – an indicator of PCR-inhibiting substances, particularly humics and polyphenols. Even samples that appear colorless may contain PCR inhibitors, which can be cleaned up using this kit. The RNeasy PowerClean Pro Cleanup Kit removes brown color as well as any PCR-inhibiting substances, such as heme, polysaccharides, polyphenols, fulvic acids and dyes. The isolated RNA has a high level of purity, which allows for more successful RT-PCR amplification. This kit has been validated with RNA isolated from a variety of problematic soils and also with RNA samples spiked with commercial humic acids. However, it performs well on RNA isolated from virtually any sample source.

Principle and procedure

Archived or previously isolated RNA samples are purified when combined with our proprietary RNA Cleanup reagents, and inhibitors are selectively removed from the RNA solution. All RNA is captured on a silica membrane in a spin column format. The RNA is then washed and eluted from the membrane. Percentage recovery may vary depending on the level of inhibitors influencing RNA yield measurements. Purified RNA is ready for RT-PCR analysis and other downstream applications.

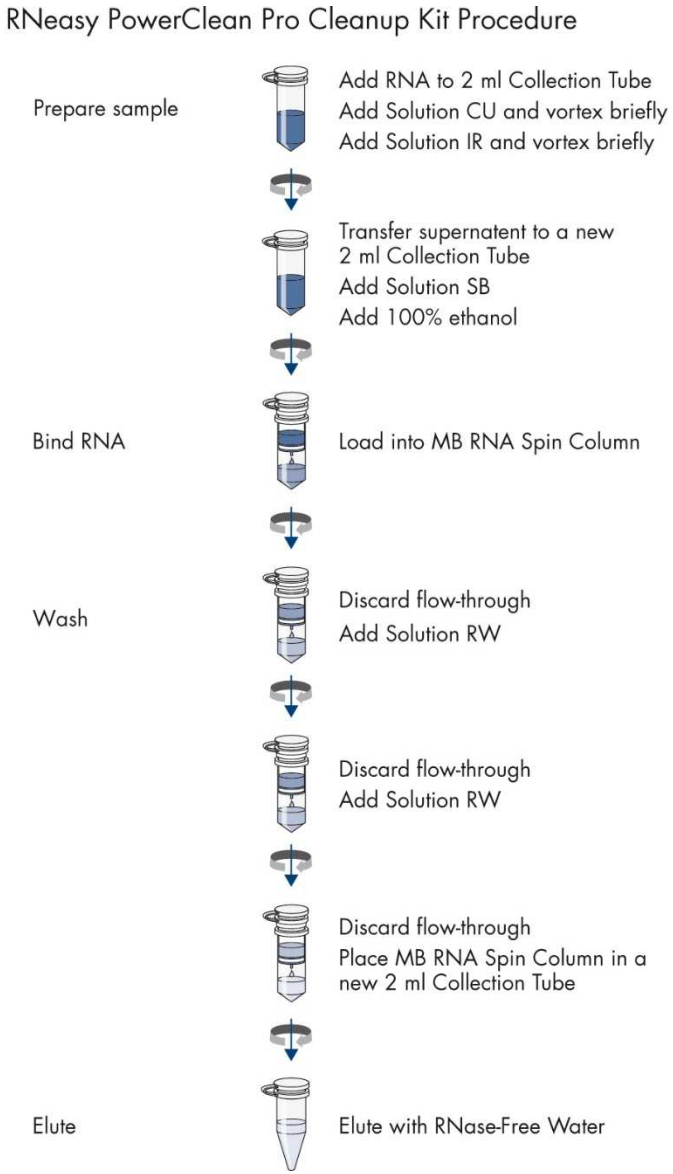

Figure 1. RNeasy PowerClean Pro Cleanup Kit procedure.

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 (up to16,000 x *g*)
- Pipettor (50–600 µl)
- Vortex-Genie® 2
- 100% ethanol

Protocol: Experienced User

Important points before starting

- Shake to mix Solution SB.
- Wear gloves at all times.

Procedure

1. Add up to 100 µl of RNA sample to a 2 ml Collection Tube (provided). If there is less than 100 µl of RNA sample, adjust the volume with RNase-Free Water (provided).
2. Add 50 µl of Solution CU and vortex briefly to mix.
3. Add 50 µl of Solution IR and vortex briefly to mix.
4. Centrifuge tubes at 13,000 x *g* for 2 min at room temperature.
5. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 160–190 µl of supernatant.
6. Shake to mix Solution SB. Add 200 µl of Solution SB and 200 µl of 100% ethanol and vortex briefly to mix.
   Note: To purify microRNAs, add an additional 200 µl of 100% ethanol and vortex briefly to mix.
7. Centrifuge tubes briefly to remove any solution from the cap.
8. Load up to 600 µl onto an MB RNA Spin Column and centrifuge at 10,000 x *g* for 1 min at room temperature. Discard the flow-through.
   Note: If 200 µl of 100% ethanol was added to recover microRNAs (in Step 6), repeat Step 8 with the remaining volume of liquid.
9. Add 500 µl of Solution RW to the MB RNA Spin Column and centrifuge at 10,000 x *g* for 30 s at room temperature. Discard the flow-through.

10. Add another 500 µl of Solution RW to the MB RNA Spin Column and centrifuge at 10,000 x *g* for 30 s at room temperature. Discard the flow-through.
11. Centrifuge the MB RNA Spin Column at maximum speed for 2 min at room temperature to remove any residual ethanol.
12. Carefully place the MB RNA Spin Column in new 2 ml Collection Tube (provided). Avoid splashing any Solution RW onto the MB RNA Spin Column.
13. To elute the RNA, add 50–100 µl of RNase-Free Water (provided) to the center of the white filter membrane. Incubate for 1 min at room temperature.
    Note: For efficient elution, use a **minimum** of 50 µl of RNase-Free Water.
14. Centrifuge at 10,000 x *g* for 1 min at room temperature.
15. Discard the MB RNA Spin Column. The RNA is now ready for downstream applications and may be stored at –65°C to –80°C.

Protocol: Detailed

Important points before starting

- Shake to mix Solution SB.
- Wear gloves at all times.

Procedure

1. Add up to 100 µl of RNA sample to a 2 ml Collection Tube (provided). If there is less than 100 µl of RNA sample, adjust the volume with RNase-Free Water (provided).
   Note: After the sample has been added to the Collection Tube, a disassociation procedure will be performed. The solutions in the RNeasy PowerClean Pro Cleanup Kit contain reagents that will (a) help disperse molecular interactions, (b) begin to dissolve humic substances and (c) protect nucleic acids from degradation.
2. Add 50 µl of Solution CU and vortex briefly to mix.
   Note: Brief vortexing mixes the components in the tube and begins to disassociate RNA from PCR-inhibiting substances.
3. Add 50 µl of Solution IR and vortex briefly to mix.
   Note: Solution IR contains Inhibitor Removal Technology (IRT): reagents that precipitate non-RNA organic and inorganic materials, including humic substances and proteins. It is important to remove contaminating organic and inorganic matter that may reduce RNA purity and inhibit downstream RNA applications.
4. Centrifuge tubes at 13,000 x *g* for 2 min at room temperature.
5. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect 160–190 µl of supernatant.
   Note: The pellet contains non-RNA organic and inorganic materials, including humic substances and proteins. For the best RNA yield and quality, avoid transferring any of the pellet.

6. Shake to mix Solution SB. Add 200 µl of Solution SB and 200 µl of 100% ethanol and vortex briefly to mix.
   Note: To purify microRNAs, add an additional 200 µl of 100% ethanol and vortex briefly to mix.
   Note: RNA binds tightly to silica at high salt concentrations in the presence of 100% ethanol. Solution SB has a high salt concentration, which allows binding of RNA, but not non-RNA organic and inorganic material that may still be present at low levels, to MB RNA Spin Columns when 100% ethanol is also present.

7. Centrifuge tubes briefly to remove any solution from the cap.

8. Load up to 600 µl onto an MB RNA Spin Column and centrifuge at 10,000 x *g* for 1 min at room temperature. Discard the flow-through.
   Note: If 200 µl of 100% ethanol was added to recover microRNAs (in Step 6), repeat Step 8 with the remaining volume of liquid.
   Note: RNA is selectively bound to the silica membrane in the MB RNA Spin Column in the presence of high salt solution/ethanol. Contaminants pass through the filter membrane, leaving only RNA bound to it.

9. Add 500 µl of Solution RW to the MB RNA Spin Column and centrifuge at 10,000 x *g* for 30 s at room temperature. Discard the flow-through.

10. Add another 500 µl of Solution RW to the MB RNA Spin Column and centrifuge at 10,000 x *g* for 30 s at room temperature. Discard the flow-through.
    Note: Solution RW is an ethanol-based wash solution used to further clean the RNA that is bound to the silica filter membrane in the MB RNA Spin Column. Solution RW removes residues of salt, humic substances and other contaminants while allowing the RNA to stay bound to the silica membrane.

11. Centrifuge the MB RNA Spin Column at maximum speed for 2 min at room temperature to remove any residual ethanol.
    Note: This drying spin removes residual Solution RW, which contains ethanol. It is critical to remove all traces of Solution RW because ethanol can interfere with several downstream applications such as PCR, restriction digests and gel electrophoresis.

12. Carefully place the MB RNA Spin Column in new 2 ml Collection Tube (provided). Avoid splashing any Solution RW onto the MB RNA Spin Column.

13. To elute the RNA, add 50–100 µl of RNase-Free Water (provided) to the center of the white filter membrane. Incubate for 1 min at room temperature.
    Note: For efficient elution, use a **minimum** of 50 µl of RNase-Free Water.
    Note: As the RNase-Free Water passes through the silica membrane of the MB RNA Spin Column, the RNA is released because it only stays bound to the silica membrane in the presence of high concentration of salt.
    Note: Placing RNase-Free Water in the center of the small white membrane will make sure the entire membrane is wet. This will result in more efficient release of RNA from the MB RNA Spin Column silica membrane.

14. Centrifuge at 10,000 x *g* for 1 min at room temperature.

15. Discard the MB RNA Spin Column. The RNA is now ready for downstream applications and may be stored at –65°C to –80°C.

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
Sample Processing/RNA		
a)	Amount of RNA to process	This kit is designed to process up to 100 µl of RNA (20 µg maximum). For inquiries about using larger amounts of sample, please contact QIAGEN Technical Services.
b)	RNA does not amplify	Check RNA yields by gel electrophoresis or spectrophotometer reading. Template RNA concentrations and other reaction conditions, such as enzyme activity and copy number of the target sequence, can influence RT-PCR outcomes. If RNA does not amplify after altering the concentration of template RNA, please contact QIAGEN Technical Services.
c)	Eluted sample is brown	We have not observed any coloration in RNA isolated using the RNeasy PowerClean Pro Cleanup Kit. If you observe coloration in your samples, please contact QIAGEN Technical Services.
d)	RNA floats out of well when loading a gel	This usually occurs because residual ethanol remains in the final sample. Avoid transferring any Solution RW to the elution step. Ethanol precipitation (described in "Concentrating eluted RNA") is the best way to remove residual ethanol.
e)	Concentrating eluted RNA	The final volume of eluted RNA will be 50–100 µl. The RNA may be concentrated by adding 5–10 µl of 3 M NaCl and inverting 3–5 times to mix. Next, add 250 µl of 100% cold ethanol and invert 3–5 times to mix. Incubate at –20°C for 20 minutes and centrifuge at 16,000 x *g* for 20 minutes at room temperature. Decant all liquid. Remove all residual ethanol in a speed vac, dessicator or ambient air. Resuspend precipitated RNA in desired volume of RNase-Free water.
f)	Storing RNA	RNA is eluted in RNase-Free Water (provided) and must be stored at –65°C to –90°C to prevent degradation.

Ordering Information

Product	Contents	Cat. no.
RNeasy PowerClean Pro Cleanup Kit (50)	For 50 preps: Remove PCR inhibitors from purified RNA in just 7 minutes	13997-50
Related products		
DNeasy PowerClean Pro Cleanup Kit (50)	For 50 preps: Remove PCR inhibitors from purified DNA in just 7 minutes	12997-50
RNeasy PowerSoil® Total RNA Kit (50)	For 25 preps: Isolate high-quality total RNA from all soil types	12866-25
RNeasy PowerPlant® Kit (50)	For 50 preps: Isolate total RNA from plant and seed samples, including those high in polyphenols and polysaccharides	13500-50
RNeasy PowerLyzer® Tissue&Cells Kit (50)	For 50 preps: Isolate total RNA from animal tissues or cells, optimized for use with bead-based homogenizers	15055-50
RNeasy PowerWater® Kit (50)	For 50 preps: Isolate total RNA from filtered water samples, including turbid water	14700-50-NF

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.

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Limited License Agreement for RNeasy PowerClean Pro Cleanup Kit

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

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