# PowerClean® Pro RNA Clean-Up Kit

| Catalog No. | Quantity |
|---|---|
| 13997-50 | 50 Preps |

## *Instruction Manual*



**Please recycle**

Version: 11152013

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## Introduction

The PowerClean® Pro RNA Clean-Up Kit utilizes our patented 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 with this kit. The PowerClean® Pro RNA Clean-Up Kit will remove this brown color as well as any PCR inhibiting substances, such as heme, polysaccharides, polyphenols fulvic acids and dyes. A high purity of RNA is achieved allowing for more successful RT-PCR amplification of RNA derived from organisms in the original sample. This kit was 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.

## Protocol Overview

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

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

| Other Related Products | Catalog No. | Quantity |
|---|---|---|
| PowerClean® Pro DNA Clean-Up Kit | 12997-50 | 50 preps |
| DEPC Treated Water | 17011-200<br>17011-5200 | 200 ml<br>5 x 200 ml |

# PowerClean® Pro RNA Isolation Kit

**Prepare Sample**

-Add RNA to 2 ml Collection Tube
- Add Solution RC1 and vortex briefly
- Add Solution RC2 and vortex briefly

*Centrifuge*

- Transfer supernatant to a new 2 ml Collection Tube
- Add Solution RC3
- Add 100% Ethanol

*Centrifuge briefly*

**Bind RNA**

- Load into Spin Filter

*Centrifuge*

**Wash**

- Discard flow through
- Add Solution RC4

*Centrifuge*

- Discard flow through
- Add Solution RC4

*Centrifuge*

- Discard flow through
- Place Spin Filter in a new 2 ml Collection Tube
- Add Solution RC5
- Incubate 1 minute

**Elute**

*Centrifuge*

## Equipment Required

Microcentrifuge (16,000 x *g*)
Pipettor (50 µl - 600 µl)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)

## Reagents Required but not Included

100% Ethanol

## Kit Contents

| | **Kit Catalog #13997-50** | |
|---|---|---|
| **Component** | **Catalog #** | **Amount** |
| Solution RC1 | 13997-50-1 | 3 ml |
| Solution RC2 | 13997-50-2 | 3 ml |
| Solution RC3 | 13997-50-3 | 11 ml |
| Solution RC4 | 13997-50-4 | 2 x 28 ml |
| Solution RC5 | 13997-50-5 | 11 ml |
| Spin Filters | 13997-50-SF | 50 |
| 2 ml Collection Tubes | 13997-50-T | 150 |

## 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 RC4 contains ethanol. It is flammable.

**IMPORTANT NOTE FOR USE:** Shake Solution RC3 before use.

# Experienced User Protocol

**Please wear gloves at all times**

1. Add up to 100 µl of RNA sample to **2 ml Collection Tube** (provided). If less than 100 µl is added, adjust the volume to 100 µl with **Solution RC5**.

2. Add 50 µl of **Solution RC1** to RNA. Vortex briefly to mix.

3. Add 50 µl of **Solution RC2** and vortex briefly to mix.

4. Centrifuge tubes at 13,000 x *g* for 2 minutes at room temperature.

5. Avoiding pellet, transfer the entire volume (expect 160 – 190 µl) of supernatant to a clean **2 ml Collection Tube** (provided).

6. Shake to mix **Solution RC3**. Add 200 µl of **Solution RC3** and 200 µl of 100% Ethanol and vortex briefly to mix.
   **Note:** If micro RNA is a component of the sample, 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 the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard flow through.
   **Note:** If the additional 200 µl was added to recover the micro RNA, a second loading of the spin filter with the remaining volume will be required.

9. Add 500 µl of **Solution RC4** to the **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

10. Once again add 500 µl of **Solution RC4** to the **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

11. Centrifuge the **Spin Filter** at maximum speed for 2 minutes at room temperature to remove any residual ethanol from the wash in steps 9 & 10.

12. Carefully place the **Spin Filter** in new **2 ml Collection Tube** (provided). Avoid splashing any **Solution RC4** onto the **Spin Filter**.

13. To elute the RNA, add the desired final volume between 50-100 µl of **RNA Solution RC5** (RNase-Free water) to the center of white filter membrane. Incubate 1 minute at room temperature. Centrifuge at 10,000 x *g* for 1 minute at room temperature.
    **Note:** For efficient recovery, use a minimum of 50 µl of **Solution RC5**.

14. Discard the **Spin Filter**. RNA in **2 ml Collection Tube** is now application ready. No further steps are required. We recommend storing RNA frozen at -80°C.

**Thank you for choosing the PowerClean® Pro RNA Clean-Up Kit.**

## Detailed Protocol (Describes what is happening at each step)

**Please wear gloves at all times**

1. Add up to 100 µl of RNA sample to a **2 ml Collection Tube** (provided). If less than 100 µl is added, adjust the volume with **Solution RC5**.

   *What's happening: After the sample has been added to the Collection Tube, a disassociation procedure is performed. The PowerClean®Pro RNA Solutions 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 RC1** to RNA. Vortex briefly to mix.

   *What's happening: Brief vortexing mixes the components in the tube and begins to disassociate RNA from PCR inhibiting substances.*

3. Add 50 µl of **Solution RC2** and vortex briefly to mix.

   *What's happening: Solution RC2 is patented Inhibitor Removal Technology® (IRT). It contains 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 the tube at 13,000 x *g* for 2 minutes at room temperature.

5. Avoiding pellet, transfer the entire volume (expect 160 – 190 µl) of supernatant to a clean **2 ml Collection Tube** (provided).

   *What's happening: 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 RC3**. Add 200 µl of **RNA Solution RC3** and 200 µl of 100% Ethanol and vortex briefly to mix.

   **Note:** If micro RNA is a component of the sample, add an additional 200 µl of 100% Ethanol and vortex briefly to mix.

   *What's happening: Solution RC3 is a high salt concentration solution. Since RNA binds tightly to silica at high salt concentrations in conjunction with 100% ethanol, this solution will adjust the salt concentrations to allow binding of RNA to the Spin Filters, but not non-RNA organic and inorganic material that may still be present at low levels.*

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

8. Load up to 600 µl onto **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard flow through.

   **Note:** If the additional 200 µl was added to recover the micro RNA, a second loading of the spin filter with the remaining volume will be required.

   *What's happening: RNA is selectively bound to the silica membrane in the Spin Filter device in the high salt/ethanol solution. Contaminants pass through the filter membrane, leaving only the RNA bound to the membrane.*

9. Add 500 µl of **Solution RC4** to the **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

10. Once again add 500 µl of **Solution RC4** to the **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds at room temperature. Discard flow through.

    *What's happening: This solution is an ethanol based wash solution used to further clean the RNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residues of salt, humic substances, and other contaminants while allowing the RNA to stay bound to the silica membrane.*

11. Centrifuge the **Spin Filter** at maximum speed for 2 minutes at room temperature to remove any residual ethanol from the wash in steps 9 & 10.

    *What's happening: This "drying" spin removes residual ethanol wash solution. It is critical to remove all traces of wash solution because the ethanol in Solution RC4 can interfere with many downstream applications such as PCR and gel electrophoresis.*

12. Carefully place the **Spin Filter** in new **2 ml Collection Tube** (provided). Avoid splashing any **Solution RC4** onto the **Spin Filter**.

    ***Note:*** *It is important to avoid any traces of the ethanol based wash solution.*

13. To elute the RNA, add the desired final volume between 50-100 µl of **Solution RC5** (RNase-Free water) to center of white filter membrane. Incubate 1 minute at room temperature. Centrifuge at 10,000 x *g* for 1 minute at room temperature.
    **Note:** For efficient recovery, use a minimum of 50 µl of **Solution RC5**.

    *What's happening: As Solution RC5 (RNase-Free water) passes through the silica membrane, RNA is released because it only stays bound to the silica Spin Filter membrane in the presence of high concentrations of salt /ethanol.*

14. Discard the **Spin Filter**. The RNA in the **2 ml Collection Tube** is now application ready. No further steps are required. We recommend storing RNA frozen at -80°C.

**Thank you for choosing the PowerClean® Pro RNA Clean-Up Kit.**

# Hints and Troubleshooting Guide

***Amount of RNA to Process***
This kit is designed to process up to 100 µl of RNA (20 µg maximum). For inquiries regarding the use of larger sample amounts, please contact technical support for suggestions.

***If RNA Does Not Amplify***
Make sure to check RNA yields by gel electrophoresis or spectrophotometer reading. Template RNA concentration could influence the outcome of RT-PCR along with other reaction conditions, enzyme activity, and copy number of the target sequence. If RNA does not amplify after altering the concentration of template RNA, please call our technical support for suggestions.

***Eluted RNA Sample Is Brown***
We have not observed any coloration in RNA isolated using the PowerClean® Pro RNA Clean-Up Kit. If you observe coloration in your samples, please contact technical support for suggestions.

***Concentrating the RNA***
The final volume of eluted RNA will be up to 100 µl depending on the amount of starting material. The RNA may be concentrated by adding 1/10$^{th}$ volume of 5 M NaCl and inverting 3-5 times to mix. Next, add 250 µl of 100% cold ethanol and mix. Incubate at -20°C for 20 minutes. Centrifuge at 16,000 x *g* for 20 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac, dessicator, or ambient air. Resuspend precipitated RNA in RNase-Free water.

***RNA Floats Out of Well When Loaded on a Gel***
Residual Solution RC4 remains in the final sample. Prevent this by being careful not to transfer liquid onto the bottom of the Spin Filter basket. Ethanol precipitation is the best way to remove residual Solution RC4. (See “Concentrating the RNA” above)

***Storing RNA***
RNA is eluted in Solution RC5 (RNase-Free water) and must be stored at -80°C to prevent degradation.

***Technical Tips***
Visit MO BIO’s *The Culture Dish* at http://www.mobio.com/blog/ for the latest in technical tips for frequently asked questions. Use this valuable resource to share your suggestions and optimization techniques for difficult or problematic samples.

## Contact Information

**Technical Support:**
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**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 |
|---|---|---|
| PowerClean® Pro DNA Clean-Up Kit | 12997-50 | 50 preps |
| DEPC Treated Water | 17011-200<br>17011-5200 | 200 ml<br>5 x 200 ml |
| RNA PowerSoil® Total RNA Isolation Kit | 12866-25 | 25 preps |
| PowerPlant® RNA Isolation Kit | 13500-50 | 50 preps |
| PowerPlant® RNA Isolation Kit with DNase | 13550-50 | 50 preps |
| RTS DNase™ Kit | 15200-50 | 50 preps |
| PowerMicrobiome™ RNA Isolation Kit | 26000-50 | 50 preps |