



PowerViral™ Environmental RNA/DNA Isolation Kit

Catalog No.	Quantity
28000-50	50 Preps
28000-BUNDLE	50 Preps + Bead Tubes

Instruction Manual

Inhibitor Removal Technology® (IRT) is a registered trademark of MO BIO Laboratories, Inc. and is covered by the following patents USA US 7,459,548 B2, Australia 2005323451, Japan 5112064 and India 246946.



Please recycle

Version: 07192013

Technical Information: Toll free 1-800-606-6246, or 1-760-929-9911 Email: technical@mobio.com Website: www.mobio.com



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Introduction

The PowerViral™ Environmental RNA/DNA Isolation Kit is designed for fast and easy purification of viral and microbial total nucleic acids from samples high in PCR inhibitors; including waste water, biosolids, and gut material. Patented Inhibitor Removal Technology® (IRT) ensures complete removal of the inhibitory substances often contained in these materials, such as undigested plant material in the gut or heme compounds from lysed red blood cells, abundant in stool. The result is DNA and RNA that is ready to use in the most demanding downstream applications. Nucleic acids are eluted in RNase-Free Water and ready for PCR, cDNA synthesis and RT-qPCR.

Protocol Overview

Cell Lysis Guide

There are a few optional steps that you may choose from depending on your sample type and desired cell lysis method. Please use the guide below to decide on the exact protocol you will follow.

- If you want to isolate RNA, you will need to prepare a β -mercaptoethanol solution we call Solution PV1/ β ME before you start. This solution is compatible with both the bead beating lysis method and the phenol:chloroform lysis method described below.
- There are two ways to incorporate the use of this solution. You can either prepare a pre-mix as described in the **Important Notes Before Starting on page 6**, or you can add β -mercaptoethanol and Solution PV1 separately to each sample tube. We recommend the pre-mix.
- For cell lysis, bead beating is optional. It should be used when lysis of microbial cells is desired or if the starting sample contains solid material that needs dispersing. The 0.1 mm Glass Bead Tubes are included in Catalog# 28000-BUNDLE.
- If a phenol-based lysis is desired, add 100 μ l of phenol:chloroform:isoamyl alcohol pH 6.5-8.0 to the Bead Tube before adding the sample.
- For the purification of small RNAs, such as microRNA and siRNA, Step 11 requires adding additional ethanol to enhance binding these small RNA.

Amount of Sample to Process

For liquid samples such as viral concentrate, extract, fecal suspension or waste water, the recommended starting volume is 200 μ l. For biosolid material or stool, 0.25 grams is suggested. Stool samples must be stored at -80°C as soon as possible after collection to protect the integrity of the RNA.

Sample Type

For stool samples or samples with a solid matrix, lysis is achieved using 0.1 mm Glass Bead Tubes in combination with a strong chemical lysis buffer that ensures efficient extraction of tough microorganisms in the bead beating step. For virus extraction from water samples and samples that do not require dispersion, the bead beating step may be skipped and virus extracted through chemical lysis only. The binding solution (PV3) captures the total nucleic acid content in the lysate. The RNA is eluted in RNase-Free Water and is ready to use in RT-PCR.

Bead Tubes

If lysis with Glass Bead Tubes is required, purchase Catalog# 28000-BUNDLE, which contains the PowerViral™ Environmental RNA/DNA Isolation Kit plus 0.1 mm Glass Bead Tubes. Otherwise, purchase Catalog# 28000-50 which is the PowerViral™ Environmental RNA/DNA Isolation Kit alone.



Protocol Overview cont.

DNase

If additional DNase treatment is required, we recommend the RTS DNase™ Kit (Catalog# 15200-50), a novel method for removing genomic DNA using a room temperature stable DNase enzyme. With RTS DNase, up to 30 µg of DNA is removed in 20 minutes and the DNase enzyme is easily removed using a proprietary resin. No heat or EDTA is required to inactivate the DNase. RNA retains its original quality and stability when treated with the RTS DNase™ Kit.

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

Other Related Products	Catalog No.	Quantity
RTS DNase™ Kit	15200-50	50 preps
Vortex Adapter for 2 ml tubes	13000-V1-24	1 unit

Storage of Virus Concentrates and Samples

Viral concentrates need to be stored at -80°C for long term storage. Viral concentrates may be maintained at -20°C for a few days to weeks. Viruses will degrade after several months at -20°C. It is also recommended to aliquot the virus before freezing so that the sample does not degrade during the freeze thaw cycle.

Biosolid and Stool Sample Storage and Preservation

Stool or biosolid material should be stored at -80°C for long term preservation before nucleic acid isolation. The yield and integrity of the RNA isolated from microbes and virus in biosolids or stool is greatly influenced by many factors including the length of time between collection of the sample and preservation at -80°C. The main components of stool are water (between 65%-85%), undigested food, bile, bilirubin (which is derived from dead red blood cells), and dead bacterial cells (up to 50%). Because of the high content of dead and decaying bacterial and human cells, RNA isolated from stool will typically appear to have some level of degradation using standard analysis methods. Quantitative PCR assays developed for RNA species from stool or gut should be designed to detect small fragments, to increase detection sensitivity.

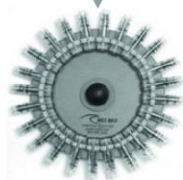
PowerViral™ Environmental RNA/DNA Isolation Kit

Prepare Sample



- Add sample to 0.1 mm Glass Bead Tube, if bead beating, or 2 ml collection tube if bead beating is not required
- Add Solution PV1/βME

Cell Lysis



- If bead beating, attach to vortex adapter and vortex
- For samples in the 2 ml collection tube, vortex to mix and incubate at room temperature

Centrifuge

Inhibitor Removal Technology®



- Add Solution PV2
- Incubate at 4°C

Centrifuge

Bind RNA and DNA



- Add Solution PV3 and PV4
- Load into Spin Filter

Centrifuge

Wash



- Wash with Solution PV5

Centrifuge

Wash



- Wash with Solution PV4

Centrifuge

Elute



- Elute with Solution PV6



Equipment Required

Microcentrifuge (13,000 x g)

Pipettors

Vortex-Genie[®] 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)

Vortex Adapter (MO BIO Catalog# 13000-V1-24)

Optional Reagents

β -Mercaptoethanol (β ME) (for RNA purification)

Phenol:Chloroform:Isoamyl Alcohol (25:24:1) pH 6.7-8.0 (Amresco Catalog# 0883-100, 0883-400)

www.amresco-inc.com or (US) 800.829.2802

Kit Contents

Kit Catalog# 28000-50		
Components	Catalog #	Amount
Solution PV1	28000-50-1	32 ml
Solution PV2	28000-50-2	11 ml
Solution PV3	28000-50-3	32 ml
Solution PV4	28000-50-4	2 x 30 ml
Solution PV5	28000-50-5	30 ml
Solution PV6	28000-50-6	5.5 ml
Spin Filters	28000-50-SF	50
2.2 ml Lysate Tubes	28000-50-LT	50
2 ml Collection Tubes	28000-50-T	200

**Kit Catalog # 28000-BUNDLE includes all items listed above plus 0.1 mm Glass Bead Tubes, Catalog #13118-50 (50 tubes).

Kit Storage

Store all reagents and kit components 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: Solutions PV4 and PV5 contain alcohol. They are flammable.

Important Notes Before Starting

For RNA Isolation, prepare Solution PV1/ β ME by adding β -mercaptoethanol (β ME)

Add enough β -mercaptoethanol (β ME) to Solution PV1 to produce a final concentration of 10 μ l/ml. For best results do not use the PV1/ β ME mixture if greater than a month old. Since the PV1/ β ME mixture loses its effectiveness over time, it's best to make it fresh each time. For each prep, you will need 600 μ l of PV1/ β ME solution. Alternatively, you may add 600 μ l of solution PM1 and 6 μ l β ME to each sample. β ME may be optional for clean sample types. If you choose to not use β ME, we recommend performing an evaluation to determine the results prior to performing large scale studies.

Note: Use a fume hood when opening β ME to avoid exposure to the chemical.



Experienced User Protocol

Please see Important Notes Before Starting on page 6. Please wear gloves at all times.

Warm Solution PV1 at 55°C for 10 minutes prior to use to dissolve precipitates. Use Solution PV1 while still warm. Shake to mix before using.

Important: Use of bead beating is optional but should be used when lysis of microbial cells is desired or if the starting sample contains solid material that needs dispersing. For viral nucleic acid isolation from liquid samples, bead beating is generally not required. The 0.1 mm Glass Bead Tubes are included in Catalog# 28000-BUNDLE.

Note: β -mercaptoethanol (β ME) is only necessary for RNA isolation.

1. If bead beating is not required, 200 μ l of viral concentrate or water may be placed into a 2 ml Collection Tube (provided). If using bead beating, skip to step 3.
2. Add **600 μ l of Solution PV1/ β ME** (see **Important Notes before Starting** section) to the 2 ml Collection Tube. Alternatively, you may add separately 600 μ l PV1 and 6 μ l of β ME to the Collection Tube. Vortex the viral concentrate and PV1/ β ME for 30 seconds to mix and then incubate the sample for 5 minutes at room temperature. **Go to step 8.**
3. If bead beating is required (see recommendations above) add either 0.25 grams of stool/biosolid or 200 μ l of liquid into the 0.1 mm Glass Bead Tube provided in Catalog# 28000-BUNDLE.
Note: If a phenol-based lysis is desired, add 100 μ l of phenol:chloroform:isoamyl alcohol pH 6.5-8.0 to the Bead Tube before adding the sample.
4. Add **600 μ l of Solution PV1/ β ME** (see **Important Notes before Starting** section) to the 0.1 mm Glass Bead Tube. Alternatively, you may add separately 600 μ l PV1 and 6 μ l of β ME to the Tube.
5. Secure the bead tubes horizontally to a MO BIO Vortex Adapter (Catalog# 13000-V1-24). The tube caps should be oriented pointing toward the center of the Vortex Adapter.
6. Vortex at maximum speed for 10 minutes.
7. Centrifuge at 13,000 x *g* for 1 minute at room temperature. Transfer the supernatant to a clean 2 ml Collection Tube (provided). The expected volume is ~600 μ l. If you added phenol:chloroform: isoamyl alcohol, remove the upper aqueous layer and transfer to a clean 2 ml Collection Tube.
8. Add **150 μ l of Solution PV2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
9. Centrifuge the tubes at 13,000 x *g* for 1 minute.
10. Avoiding the pellet, transfer the supernatant to a clean **2.2 ml Lysate Tube**. Do not transfer more than 700 μ l at this step.
11. Add **600 μ l of Solution PV3** and **600 μ l of Solution PV4**. Vortex briefly to mix.

Note: For the purification of small RNAs, such as microRNA and siRNA, transfer the lysate to a larger tube to accommodate a higher volume (2.5 ml) and add an additional 600 µl of 100% ethanol to the lysate. You will need to supply 100% ethanol for this step.

12. Load 625 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.

Note: A total of three loads for each sample processed is required and four loads if an additional volume of 100% ethanol is added for the microRNA and siRNA protocol.

13. Shake to mix Solution PV5. Add **600 µl of Solution PV5** to the Spin Filter and centrifuge at 13,000 x *g* for 1 minute.

14. Discard the flow through and add **600 µl of Solution PV4** and centrifuge at 13,000 x *g* for 1 minute.

15. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove any residual wash solution.

16. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

17. Add **100 µl of Solution PV6 (RNase-Free Water)** to the center of the white filter membrane. Allow the water to sit on the membrane for at least 1 minute.

Note: Eluting with 100 µl of Solution PV6 will maximize DNA/RNA yield. For more concentrated DNA/RNA, a minimum of 50 µl of Solution PV6 can be used. Do not use less than 50 µl of Solution PV6.

18. Centrifuge at 13,000 x *g* for 1 minute.

19. Discard the Spin Filter basket.

The DNA and RNA is now ready for any downstream applications. The RNA in the tube can be stored at -80°C until use.

Thank you for choosing the PowerViral™ Environmental RNA/DNA Isolation Kit

For further removal of genomic DNA post-RNA extraction, we recommend the RTS DNase™ Kit, Catalog# 15200-50, which contains a DNase removal resin to generate DNA-free RNA without the use of heat or EDTA to inactivate enzymes.



Detailed Protocol (Describes what is happening at each step)

Please see Important Notes Before Starting on page 6. Please wear gloves at all times.

Warm Solution PV1 at 55°C for 10 minutes prior to use to dissolve precipitates. Use Solution PV1 while still warm. Shake to mix before using.

Use of bead beating is optional and should be used if lysis of microbial cells is required or if the starting sample has solid material that needs dispersing. For viral nucleic acids isolation from liquid samples, 200 µl of virus concentrate or water may be placed into a 2 ml Collection Tube. The 0.1 mm Glass Bead Tubes are included in Catalog# 28000-BUNDLE.

Note: β-mercaptoethanol (βME) is only necessary for RNA isolation.

1. If bead beating is not required, 200 µl of virus concentrate or water may be placed into a 2 ml Collection Tube (provided). If using bead beating, skip to step 3.
2. Add **600 µl of Solution PV1/βME** (see **Important Notes before Starting** section) to the 2 ml Collection Tube. Alternatively, you may add separately 600 µl PV1 and 6 µl of βME to the Collection Tube. Vortex the virus concentrate and PV1/βME for 30 seconds to mix and then incubate the sample for 5 minutes at room temperature. **Go to step 8.**

What's happening: PV1/βME is a lysis buffer that prevents RNase activity for isolation of RNA viruses. βME is not necessary for isolation of DNA from viruses and may not be needed for clear samples. Liquid samples do not require bead beating. Incubation for five minutes in lysis buffer will release the nucleic acids from virus in suspension.

3. If bead beating is required (see recommendations above) add either 0.25 grams of stool/biosolid or 200 µl of liquid into the 0.1 mm Glass Bead Tube provided in Catalog# 28000-BUNDLE.

Note: If a phenol-based lysis is desired, add 100 µl of phenol:chloroform:isoamyl alcohol pH 6.5-8.0 to the Bead Tube before adding the sample.

What's happening: Bead beating is used to disperse solid samples and to lyse microbial cells.

4. Add **600 µl of Solution PV1/βME** (see **Important Notes before Starting** section) to the 0.1 mm Glass Bead Tube. Alternatively, you may add separately 600 µl PV1 and 6 µl of βME to the Tube.
5. Secure the bead tubes horizontally to a MO BIO Vortex Adapter (Catalog# 13000-V1-24). The tube caps should be oriented pointing toward the center of the Vortex Adapter.
6. Vortex at maximum speed for 10 minutes.

What's happening: The sample is homogenized using mechanical bead beating and a lysis buffer that protects the RNA released into the supernatant. Bead beating is recommended for samples where microbial RNA and DNA are desired or for samples with solid material that requires homogenization to release virus particles.

7. Centrifuge at 13,000 x g for 1 minute at room temperature. Transfer the supernatant to a clean 2 ml Collection Tube (provided). The expected volume is ~600 µl. If you added phenol:chloroform: isoamyl alcohol, remove the upper aqueous layer and transfer to a clean 2 ml Collection Tube.

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What's happening: Proteins and cellular debris are pelleted with the beads and the supernatant contains RNA and DNA from virus or cells.

8. Add **150 µl of Solution PV2** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
9. Centrifuge the tubes at 13,000 x g for 1 minute.

What's happening: Solution PV2 is Inhibitor Removal Solution which completes the IRT process and removes the contaminants from the sample that would cause problems with PCR and other downstream applications.

10. Avoiding the pellet, transfer the supernatant to a clean **2.2 ml Lysate Tube**. Do not transfer more than 700 µl at this step.
11. Add **600 µl of Solution PV3** and **600 µl of Solution PV4**. Vortex briefly to mix.
Note: For the purification of small RNAs, such as microRNA and siRNA, transfer the lysate to a larger tube to accommodate a higher volume (2.5 ml) and add an additional 600 µl of 100% ethanol to the lysate. You will need to supply 100% ethanol for this step.

What's happening: Solution PV3 contains the binding salts for total nucleic acid purification and Solution PV4 is 100% ethanol. These solutions set up the conditions for RNA and DNA binding to the Spin Filter.

12. Load 625 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x g for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.
Note: A total of three loads for each sample processed is required and four loads if an additional volume of 100% ethanol is added for the microRNA and siRNA protocol.

What's happening: The total nucleic acids are bound to the Spin Filter by passing it through the membrane using centrifugation.

13. Shake to mix Solution PV5. Add **600 µl of Solution PV5** to the Spin Filter and centrifuge at 13,000 x g for 1 minute.
14. Discard the flow through and add **600 µl of Solution PV4** and centrifuge at 13,000 x g for 1 minute.

What's happening: Solution PV5 and PV4 are wash buffers containing isopropanol and ethanol, respectively and are used to desalt the column before the elution step.

15. Discard the flow through and centrifuge again at 13,000 x g for 2 minutes to remove any residual wash solution.

What's happening: The final dry spin ensures all of the ethanol is cleared from the membrane so that the elution will be efficient.

16. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
17. Add **100 µl of Solution PV6 (RNase-Free Water)** to the center of the white filter membrane. Allow the water to sit on the membrane for at least 1 minute.
Note: Eluting with 100 µl of Solution PV6 will maximize DNA/RNA yield. For more concentrated DNA/RNA, a minimum of 50 µl of Solution PV6 can be used. Do not use less than 50 µl of Solution PV6.



18. Centrifuge at 13,000 x *g* for 1 minute.

What's happening: RNA and DNA is solubilized from the Spin Filter membrane into Solution PV6 (RNase-Free Water) and is ready for use in enzymatic applications.

19. Discard the Spin Filter basket.

The RNA is now ready for any downstream applications. The RNA in the tube can be stored at -80°C until use.

Thank you for choosing the PowerViral™ Environmental RNA/DNA Isolation Kit

For further removal of genomic DNA post-RNA extraction, we recommend the RTS DNase™ Kit, Catalog# 15200-50, which contains a DNase removal resin to generate DNA-free RNA without the use of heat or EDTA to inactivate enzymes.

Hints and Troubleshooting Guide

RNA Degradation

The optimal method for storing stool samples prior to RNA isolation is to freeze at -80°C and to avoid multiple freeze/thaw cycles. The primary solid component of stool is dead bacteria which will contain a high level of degraded RNA. Co-isolation of degraded RNA along with intact RNA is expected.

Removal of small degraded RNA is achieved by reducing the concentration of ethanol used at the binding step (Step 10). Instead of Solution PV4, use 600 µl of 70% ethanol.

If using stool samples preserved in RNeasy[®], use only 0.1 gram of sample to avoid clogging the column. RNeasy[®] preserved samples will require additional DNase treatment post-purification to remove contaminating genomic DNA.

The use of phenol:chloroform:isoamyl alcohol (25:24:1) buffered to pH 6.7-8.0 is optional and may assist in protecting the integrity of the RNA during homogenization of the sample. Add 100 µl of phenol:chloroform:isoamyl alcohol to the 0.1 mm Glass Bead Tube before adding the sample. For most solid samples, an organic phase and an aqueous phase may not be visible. However, for watery samples you may see a separation of phases. Always take the upper aqueous phase for the next step.

Low Purity

RNA and DNA yields from viral concentrates or extracts will often have undetectable levels of nucleic acid using conventional quantification techniques (UV spectrophotometry or fluorescent dye based detection). Purity readings on the UV Spec are not reflective of the sample when yields fall below the accurate range for the instrument. For the Nanodrop this yield is <10 ng/µl. A positive control may be useful in determining the purity of the samples processed. qPCR will be more accurate for determination of the yield and purity of the samples with low biological input.

For samples with high biomass, the expected 260/280 purity ratio for RNA is 1.8-2.1 and 1.7-2.0 for DNA. The optimal ratio for the 260/230 ratio is >1.5. If the 260/230 readings are low, increase the amount of Solution PV2 to 200 µl to remove more inhibitors. Alternatively, use less sample if the purity is not improved with the addition of more PV2.

Genomic DNA contamination

If removal of genomic DNA is desired, a DNase treatment using the RTS DNase[™] Kit Catalog # 15200-50 will ensure removal of DNA without the need for addition of EDTA or heat inactivation of the enzyme.



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
PowerWater® DNA Isolation Kit	14900-50-NF	50 preps
	14900-100-NF	100 preps
PowerWater® RNA Isolation Kit	14700-50-NF	50 preps
PowerMicrobiome™ RNA Isolation Kit	26000-50	50 preps
PowerFecal™ DNA Isolation Kit	12830-50	50 preps
RTS DNase™ Kit	15200-50	50 preps
Vortex-Genie® 2 Vortex	13111-V	1 unit (120 V)
	13111-V-220	1 unit (220 V)
Vortex Adapter for Vortex Genie® 2	13000-V1-24	Holds 24 (2 ml) Tubes