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AllPrep® PowerViral® DNA/RNA Kit Handbook

For the isolation of viral or bacterial total nucleic acids from waste water and stool samples

Sample to Insight

QIAGEN

Contents

Kit Contents

AllPrep PowerViral DNA/RNA Kit	**(50)**
Catalog no.	**28000-50**
Number of preps	**50**
PowerBead Tubes, Glass 0.1 mm	50
MB Spin Columns	50
Solution PM1	55 ml
Solution IRS	15 ml
Solution PM3	36 ml
Solution PM4	3 x 24 ml
Solution PM5	30 ml
RNase-Free Water	10 ml
Collection Tubes (2.2 ml)	2 x 25
Collection Tubes (2 ml)	4 x 50
Quick Start Protocol	1

Storage

All reagents and components of the AllPrep PowerViral DNA/RNA Kit can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

All AllPrep 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 PM4 and Solution PM5 are flammable.**

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

Solution PM1 and Solution PM3 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 AllPrep PowerViral DNA/RNA Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The AllPrep PowerViral DNA/RNA Kit is designed for fast and easy purification of viral and microbial total nucleic acids from samples with high levels of PCR inhibitors, including waste water, biosolids and gut material. The kit uses Inhibitor Removal Technology® (IRT) to ensure 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 in stool. Nucleic acids are eluted in RNase-free water and are ready for use in demanding downstream applications, such as PCR, cDNA synthesis and RT-qPCR.

Principle and procedure

For liquid samples, such as viral concentrates or extracts, fecal suspensions or waste water, the recommended starting volume is 200 µl. For biosolid material or stool, the suggested starting amount is 0.25 g. Stool samples must be stored at –65 to –90°C as soon as possible after collection to protect the integrity of the RNA.

For stool samples or samples with a solid matrix, lysis is achieved using PowerBead Tubes, Glass 0.1 mm (provided) 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.

Solution PM3 leads to the capture of the total nucleic acid content in lysates on the MB Spin Column filter membrane. Nucleic acids are eluted in RNase-free water and are ready to use in downstream applications.

If isolating RNA, prepare a mixture of β-mercaptoethanol (β-ME) and Solution PM1. Refer to the Important points before starting section (pages 10 and 12) for more information.

If phenol-based lysis is desired, add 100 µl of phenol:chloroform:isoamyl alcohol (pH 6.5–8) to each PowerBead Tube before adding sample.

To purify small RNAs, such as microRNAs and siRNAs, add ethanol (user provided) at step 10 of the protocol (pages 11 and 13).

To remove genomic DNA contamination using a high-activity DNase I enzyme and a highly specific DNase removal resin, we recommend the DNase Max® Kit (cat. no. 15200-50).

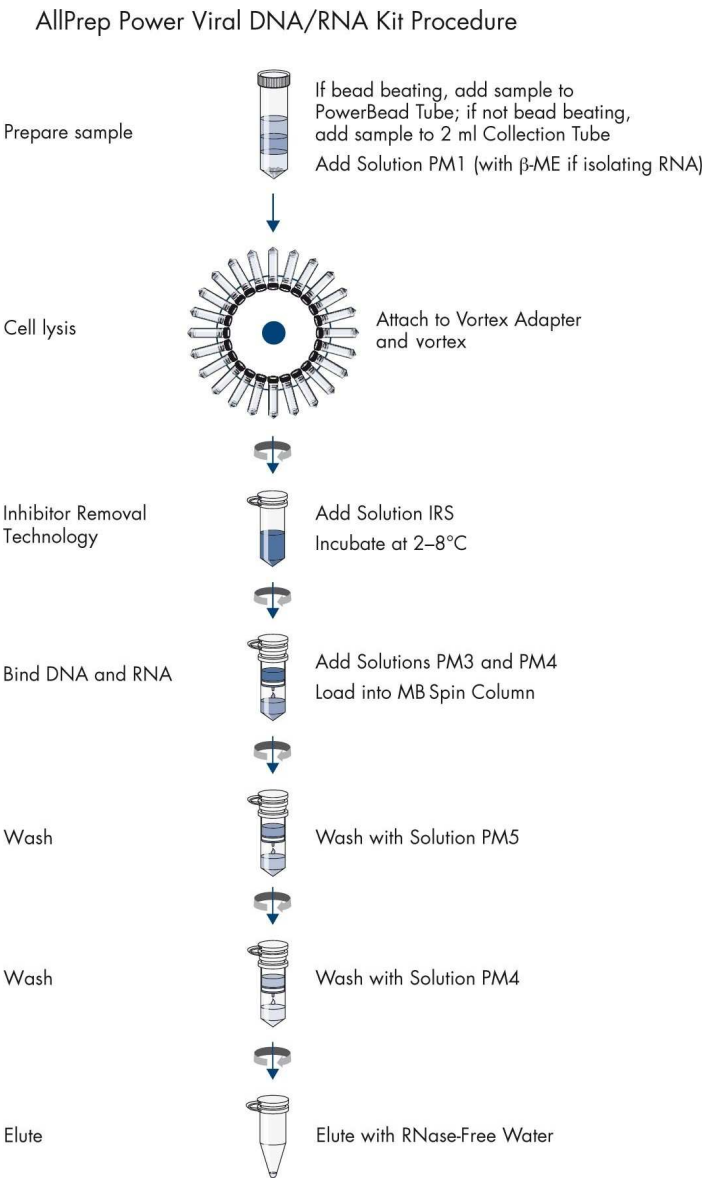

Figure 1. AllPrep PowerViral DNA/RNA Kit procedure.

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 to13,000 x *g*)
- Pipettors
- Vortex-Genie® 2
- Vortex Adapter for vortexing 1.7 or 2 ml tubes (cat. no. 13000-V1-24)
- β-mercaptoethanol (if isolating RNA)
- 100% ethanol (if isolating small RNAs, such as microRNAs and siRNAs)
- **Optional:** Phenol:chloroform:isoamyl alcohol (pH 6.5–8)

Protocol: Experienced User

Important points before starting

- Warm Solution PM1 at 55°C for 10 min prior to use to dissolve precipitates. Use Solution PM1 while still warm. Shake to mix before using.
- For RNA isolation, add β-mercaptoethanol (β-ME) to Solution PM1 to produce a mixture of Solution PM1/β-ME with a final β-ME concentration of 10 µl/ml. The mixture of Solution PM1/β-ME loses its effectiveness over time, so prepare a fresh batch each time you use the kit. You will need 600 µl of the Solution PM1/β-ME mixture per prep.
- 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.

Procedure

1. If bead beating is not required, place 200 µl of viral concentrate or water into a 2 ml Collection Tube (provided). If using bead beating, skip to Step 3.
2. Add 600 µl of the Solution PM1/β-ME mixture (see Important points before starting, above) to the 2 ml Collection Tube. Alternatively, you may add 600 µl of Solution PM1 and 6 µl of β-ME to the Collection Tube. Vortex the viral concentrate and the mix of Solution PM1/β-ME for 30 s. Incubate for 5 min at room temperature. Go to Step 8.
3. If bead beating is required, add either 0.25 g of stool/biosolid or 200 µl of liquid into a PowerBead Tube, Glass 0.1 mm (provided).
   Note: If phenol-based lysis is desired, add 100 µl of phenol/chloroform/isoamyl alcohol (pH 6.5–8.0) to the PowerBead Tube before adding the sample.
4. Add 600 µl of the Solution PM1/β-ME mixture to the PowerBead Tube. Alternatively, you may add 600 µl of Solution PM1 and 6 µl of β-ME to the PowerBead Tube.
5. Secure the bead tubes horizontally to a Vortex Adapter (cat. no. 13000-V1-24). The tube caps should be pointing toward the center of the Vortex Adapter.

6. Vortex at maximum speed for 10 min.
7. Centrifuge at 13,000 x *g* for 1 min at room temperature. Transfer the supernatant to a clean 2 ml Collection Tube (provided). If you added phenol/chloroform/isoamyl alcohol, remove the upper aqueous layer and transfer to a clean 2 ml Collection Tube (provided).
8. Add 150 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
9. Centrifuge at 13,000 x *g* for 1 min. Avoiding the pellet, transfer the supernatant to a clean 2.2 ml Collection Tube (provided). Do not transfer more than 700 µl.
10. Add 600 µl each of Solution PM3 and Solution PM4. Vortex briefly to mix.
    Note: To purify small RNAs, such as microRNAs and siRNAs, transfer the lysate to a larger tube to accommodate a higher volume (2.5 ml) and add an additional 600 µl of 100% ethanol (user provided) to the lysate.
11. Load 625 µl of supernatant into an MB Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow through and repeat until all the supernatant has been loaded onto the MB Spin Column.
    Note: A total of three loads is required for each sample processed (four loads if an additional volume of 100% ethanol was added for the microRNA/siRNA protocol).
12. Shake to mix Solution PM5 and add 600 µl to the MB Spin Column. Centrifuge at 13,000 x *g* for 1 min.
13. Discard flow-through. Add 600 µl of Solution PM4. Centrifuge at 13,000 x *g* for 1 min.
14. Discard flow-through and centrifuge at 13,000 x *g* for 2 min.
15. Place the MB Spin Column in a clean 2 ml Collection Tube (provided).
16. Add 100 µl of RNase-Free Water (provided) to the center of the MB Spin Column membrane. Incubate for at least 1 min.
    Note: Eluting with 100 µl of RNase-Free Water will maximize DNA/RNA yield. For more concentrated DNA/RNA, a **minimum** of 50 µl of RNase-Free Water can be used.
17. Centrifuge at 13,000 x *g* for 1 min. Discard the MB Spin Column. The DNA/RNA is now ready for downstream applications. RNA can be stored at –65 to –90°C.

Protocol: Detailed

Important points before starting

- Warm Solution PM1 at 55°C for 10 min prior to use to dissolve precipitates. Use Solution PM1 while still warm. Shake to mix before using.
- For RNA isolation, add β-mercaptoethanol (β-ME) to Solution PM1 to produce a mixture of Solution PM1/β-ME with a final β-ME concentration of 10 µl/ml. The mixture of Solution PM1/β-ME loses its effectiveness over time, so prepare a fresh batch each time you use the kit. You will need 600 µl of the Solution PM1/β-ME mixture per prep.
- 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.

Procedure

1. If bead beating is not required, place 200 µl of viral concentrate or water into a 2 ml Collection Tube (provided). If using bead beating, skip to Step 3.
2. Add 600 µl of the Solution PM1/β-ME mixture (see Important points before starting, above) to the 2 ml Collection Tube. Alternatively, you may add 600 µl of Solution PM1 and 6 µl of β-ME to the Collection Tube. Vortex the viral concentrate and the mix of Solution PM1/β-ME for 30 s. Incubate for 5 min at room temperature. Go to Step 8.
   Note: The Solution PM1/β-ME mix is a lysis buffer that prevents RNase activity, which is important when isolating RNA. It is not necessary to add β-ME when isolating DNA from viruses and it may not be needed for clear samples either. Liquid samples do not require bead beating. Incubation for five minutes in lysis buffer will release nucleic acids from virus in suspension.
3. If bead beating is required, add either 0.25 g of stool/biosolid or 200 µl of liquid into a PowerBead Tube, Glass 0.1 mm (provided).
   Note: If phenol-based lysis is desired, add 100 µl of phenol/chloroform/isoamyl alcohol (pH 6.5–8.0) to the PowerBead Tube before adding the sample.

Note: Bead beating is used to disperse solid samples and to lyse microbial cells.

4. Add 600 µl of the Solution PM1/β-ME mixture to the PowerBead Tube. Alternatively, you may add 600 µl of Solution PM1 and 6 µl of β-ME to the PowerBead Tube.
5. Secure the bead tubes horizontally to a Vortex Adapter (cat. no. 13000-V1-24). The tube caps should be pointing toward the center of the Vortex Adapter.
6. Vortex at maximum speed for 10 min.
   Note: The sample is homogenized using mechanical bead beating and a lysis buffer that protects 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 min at room temperature. Transfer the supernatant to a clean 2 ml Collection Tube (provided). If you added phenol/chloroform/isoamyl alcohol, remove the upper aqueous layer and transfer to a clean 2 ml Collection Tube (provided).
   Note: Proteins and cellular debris are pelleted with the beads and the supernatant contains RNA and DNA from viruses or cells.
8. Add 150 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
9. Centrifuge at 13,000 x *g* for 1 min. Avoiding the pellet, transfer the supernatant to a clean 2.2 ml Collection Tube (provided). Do not transfer more than 700 µl.
   Note: Solution IRS is an inhibitor removal solution, which completes the Inhibitor Removal Technology process and removes contaminants that would cause problems with PCR and other downstream applications from the sample.
10. Add 600 µl each of Solution PM3 and Solution PM4. Vortex briefly to mix.
    Note: To purify small RNAs, such as microRNAs and siRNAs, transfer the lysate to a larger tube to accommodate a higher volume (2.5 ml) and add an additional 600 µl of 100% ethanol (user provided) to the lysate.
    Note: Solution PM3 contains binding salts for total nucleic acid purification and Solution PM4 is 100% ethanol. These solutions set up the conditions needed for RNA and DNA binding to the MB Spin Column.

11. Load 625 µl of supernatant into an MB Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow through and repeat until all the supernatant has been loaded onto the MB Spin Column.
    Note: A total of three loads is required for each sample processed (four loads if an additional volume of 100% ethanol was added for the microRNA/siRNA protocol).
    Note: Nucleic acids are bound to the MB Spin Column as they pass through the Spin Column membrane during centrifugation.
12. Shake to mix Solution PM5 and add 600 µl to the MB Spin Column. Centrifuge at 13,000 x *g* for 1 min.
13. Discard flow-through. Add 600 µl of Solution PM4. Centrifuge at 13,000 x *g* for 1 min.
    Note: Solution PM5 and PM4 are wash buffers containing isopropanol and ethanol, respectively, and are used to desalt the column before the elution step.
14. Discard flow-through and centrifuge at 13,000 x *g* for 2 min.
    Note: The final dry spin ensures that all of the ethanol from Solution PM4 is cleared from the MB Spin Column filter membrane, which ensures efficient elution of nucleic acids.
15. Place the MB Spin Column in a clean 2 ml Collection Tube (provided).
16. Add 100 µl of RNase-Free Water (provided) to the center of the MB Spin Column membrane. Incubate for at least 1 min.
    Note: Eluting with 100 µl of RNase-Free Water will maximize DNA/RNA yield. For more concentrated DNA/RNA, a **minimum** of 50 µl of RNase-Free Water can be used.
17. Centrifuge at 13,000 x *g* for 1 min. Discard the MB Spin Column. The DNA/RNA is now ready for downstream applications. RNA can be stored at –65 to –90°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
Nucleic acids	
a) RNA is degraded	The optimal method for storing stool samples prior to RNA isolation is to freeze at –65 to –90°C and to avoid multiple freeze/thaw cycles. The primary solid component of stool is dead bacteria, which contains a high level of degraded RNA. Co-isolation of degraded RNA along with intact RNA is expected. Small degraded RNAs can be removed by reducing the concentration of ethanol used at the binding step (Step 10). Instead of Solution PM4, use 600 µl of 70% ethanol. If using stool samples preserved in RNA*later*™ (Invitrogen™), use only 0.1 gram of sample to avoid clogging the column. Samples preserved in RNA*later* will require additional DNase treatment post-purification to remove contaminating genomic DNA. The use of phenol/chloroform/isoamyl alcohol (25:24:1; pH 6.7–8.0) is optional and may assist in protecting the integrity of RNA during homogenization of the sample. Add 100 µl of phenol/chloroform/isoamyl alcohol to PowerBead Tubes before adding 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 proceed with the upper aqueous phase.
b) Removing genomic DNA contamination	Using the DNase Max Kit (cat. no. 15200-50) will ensure removal of genomic DNA without needing the addition of EDTA or heat-inactivation of the DNase I enzyme.

		Comments and suggestions
c)	Nucleic acids have 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 using UV spectrophotometry 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 the determination of the yield and purity of samples with low biological input. For samples with high biomass, the expected 260/280 purity ratio is 1.8–2.1 for RNA 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 IRS used (in step 8) to 200 µl. Alternatively, use less sample if purity is not improved with the addition of more Solution IRS.

Ordering Information

Product	Contents	Cat. no.
AllPrep PowerViral DNA/RNA Kit (50)	For 50 preps: Isolation of viral or bacterial total nucleic acids from waste water and stool samples	28000-50
Related products		
AllPrep Bacterial DNA/RNA/Protein Kit (50)	For 50 preps: Extraction of protein and nucleic acids from bacterial cell cultures	47054
AllPrep Fungal DNA/RNA/Protein Kit (50)	For 50 preps: Extraction of protein and nucleic acids from fungal cell cultures	47154
DNase Max Kit	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
DNeasy® UltraClean® 96 Microbial Kit (384)	For 384 preps: High-throughput isolation of DNA from microbial cultures	10196-4
DNeasy UltraClean Microbial Kit (50)	For 50 preps: Isolation of high-quality DNA from microbial cultures	12224-50
DNeasy UltraClean Microbial Kit (250)	For 250 preps: Isolation of high-quality DNA from microbial cultures	12224-250
Vortex Adapter	For vortexing 1.7 ml or 2 ml tubes using the Vortex-Genie 2 Vortex	13000-V1-24

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.

Limited License Agreement for AllPrep PowerViral DNA/RNA Kit

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