April 2018

AllPrep® Bacterial DNA/RNA/Protein Kit Handbook

For the extraction of protein and nucleic acids from bacterial cell cultures

Sample to Insight

QIAGEN

Contents

Kit Contents

AllPrep Bacterial DNA/RNA/Protein Kit	**(50)**
Catalog no.	**47054**
Number of preps	**50**
PowerBead Tubes, Glass 0.1 mm	50
MB Spin Columns	3 x 50
Solution AB	36 ml
IW Solution	18 ml
RW Solution	18 ml
Solution EB	8 ml
Solution MR	2 x 7 ml
Solution EA	36 ml
Solution PE	6 ml
Solution HC	20 ml
Solution RB	20 ml
WP Solution	12 ml
RNase-Free Water	7 ml
Collection Tubes (2 ml)	6 x 50
Quick Start Protocol	1

Storage

We recommend storing the MB Spin Columns at 2–8°C. All other reagents and components of the AllPrep Bacterial DNA/RNA/Protein 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.

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

Solution EA and Solution MR 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 Bacterial DNA/RNA/Protein Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The AllPrep Bacterial DNA/RNA/Protein Kit is designed to isolate total nucleic acids and cellular proteins from bacterial cultures in a patent-pending user-friendly spin filter format. Bead beating in an optimized chaotropic formulation allows users to efficiently lyse and solubilize nucleic acids and proteins from a diverse range of gram-negative and gram-positive bacteria. The use of silica spin filters to achieve reversible, sequential immobilization of DNA, RNA and proteins greatly streamlines the nucleic acid and protein isolation process, allowing direct correlations between genes, their expression and function.

Isolated nucleic acids (gDNA, rRNA, mRNA and small RNAs) are suitable for demanding downstream applications, including PCR, qPCR, RT-PCR and next-generation sequencing (NGS). Purified proteins are suitable for 1D SDS-PAGE and mass spectrometry following in-gel trypsin digestion. Some applications, including 2D SDS-PAGE and solution phase proteolytic digestion for mass spectrometry, may require an additional detergent removal step following protein elution.

Principle and procedure

The AllPrep Bacterial DNA/RNA/Protein Kit uses a novel sequential isolation method that begins with mechanical lysis of cultured bacterial cells using 0.1 mm glass bead tubes. Nucleic acids and proteins are completely solubilized during homogenization and mixed with a DNA-binding solution. DNA is bound to a spin filter and the flow-through containing RNA and proteins is then combined with a solution that binds total RNA on a second spin filter. The final flow-through, containing denatured proteins, is combined with another novel buffer to immobilize the proteins onto a third and final silica spin filter. Each spin filter containing either immobilized nucleic acids or proteins is then washed and the immobilized analyte is eluted.

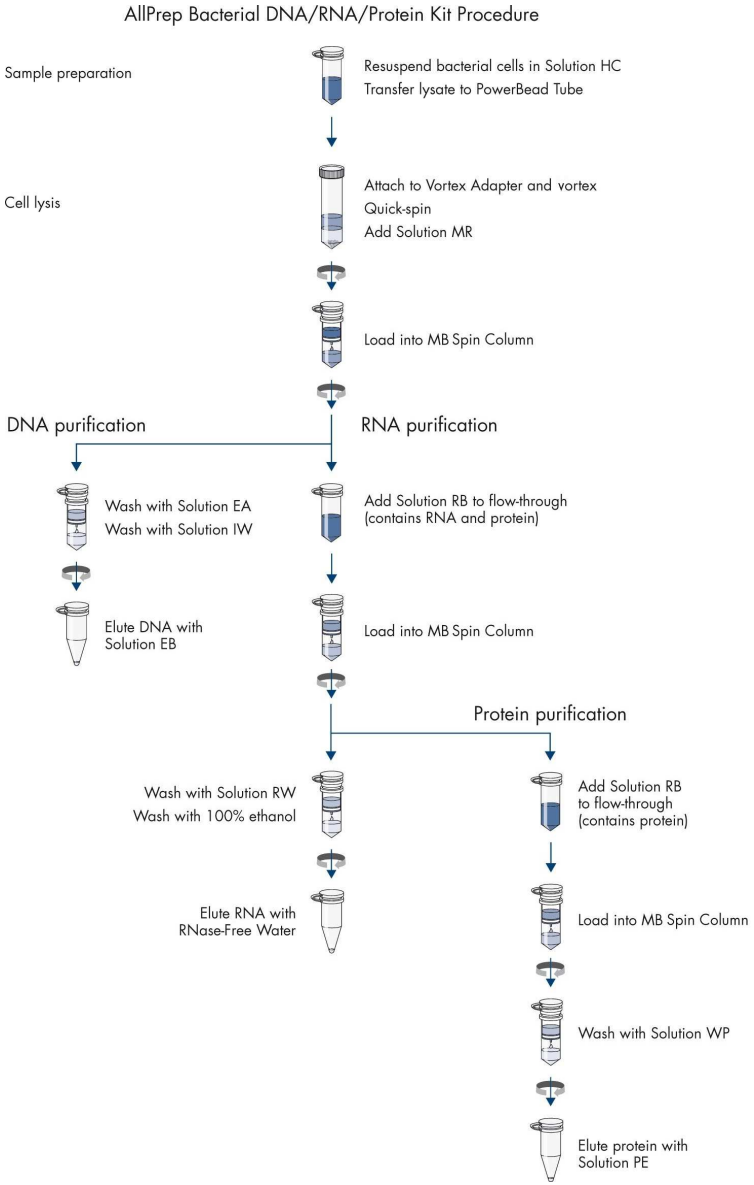

Figure 1. AllPrep Bacterial DNA/RNA/Protein Kit procedure.

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 to15,000 x *g*)
- Pipettors (1 µl–1000 µl)
- Pipette tips (1 µl–1000 µl)
- Vortex-Genie® 2
- Vortex Adapter for vortexing 1.7 or 2 ml tubes (cat. no. 13000-V1-24)
- β-mercaptoethanol (β-ME) or dithiothreitol (DTT)
- 100% ethanol
- 100% isopropanol

Protocol: Experienced User

Important points before starting

- Add 100% ethanol (user provided) to IW Solution and WP Solution as indicated on the bottle label.
- Add 100% isopropanol (user provided) to RW Solution as indicated on the bottle label.
- To prepare a working stock of Solution HC, add 3.5 µl of β-mercaptoethanol (β-ME) per 350 µl of Solution HC. Alternatively, dithiothreitol (DTT) may be added to Solution HC to a final concentration of 1–10 mM. Use a fume hood when using β-ME or DTT.
- We strongly recommend using Halt™ Protease Inhibitor Cocktail (Thermo Fisher Scientific cat. no. 78429). Use 3.5 µl of Halt Protease Inhibitor Cocktail per sample. Follow the manufacturer's recommendations when using other protease inhibitors.
- All protocol steps must be followed in the order written. DNA, RNA and protein subheadings are not stand alone protocols. Skipping steps will result in reduced binding efficiency and reduced recovery of the desired biomolecule.

Procedure

1. Add 1.8 ml of bacterial culture to a 2 ml Collection Tube (provided). Centrifuge for 3 min at 15,000 x *g*. Remove all of the supernatant with a pipette tip.
   Note: We recommend using no more than $1x10^9$ bacterial cells per sample.
2. Re-suspend the cell pellet in 350 µl of Solution HC (working stock) by vortexing or pipetting. Transfer the re-suspended cells to a PowerBead Tube.
   Note: Solution HC must be freshly prepared with β-ME (or DTT) and protease inhibitors.
3. Secure the PowerBead Tube horizontally using a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
4. Quick-spin the PowerBead Tube. Remove the cap and add 175 µl of Solution MR directly to the PowerBead Tube. Recap and vortex on high for at least 10 s to mix.
5. Centrifuge the PowerBead Tube at 15,000 x *g* for 2 min at room temperature.

DNA Purification

6. Transfer 350 µl of lysate from the PowerBead Tube directly to an MB Spin Column. Centrifuge for 1 min at 15,000 x *g*. Save flow-through for RNA purification (step 14).
   Note: It is normal to transfer some glass beads with the lysate.
7. Transfer the MB Spin Column to a clean 2 ml Collection Tube (provided).
8. Add 650 µl of Solution EA and centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
9. Add 650 µl of IW Solution and centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
10. Centrifuge at 15,000 x *g* for 2 min. Being careful not to splash liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
11. Add 100 µl of Solution EB to the center of the white filter membrane.
12. Incubate for 1 min at room temperature. Centrifuge at 15,000 x *g* for 1 min.
13. Discard the MB Spin Column. The DNA is now ready for downstream applications.

RNA purification

14. Add 350 µl of Solution RB to the flow-through from step 6. Vortex briefly on high.
15. Add the lysate to a new MB Spin Column and centrifuge at 15,000 x *g* for 1 min. Save the flow-through for protein purification (step 23).
16. Transfer the MB Spin Column to a clean 2 ml Collection Tube (provided).
17. Add 650 µl of RW Solution. Centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
18. Add 650 µl of 100% ethanol (user provided). Centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.
19. Centrifuge at 15,000 x *g* for 2 min. Being careful not to splash liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
20. Add 100 µl of RNase-Free Water (provided) to the center of the white filter membrane.
21. Incubate for 1 min at room temperature. Centrifuge at 15,000 x *g* for 1 min.
22. Discard the MB Spin Column. The RNA is now ready for downstream applications.

Protein purification

23. Add 650 µl of Solution AB to the flow-through from step 15. Vortex briefly on high.
24. Load up to 650 µl into a new MB Spin Column and centrifuge at 15,000 x *g* for 1 min.
25. Discard the flow-through and load the remaining sample volume into the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min and discard the flow-through.
26. Add 650 µl of WP Solution. Centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
27. Centrifuge at 15,000 x *g* for 2 min. Being careful not to splash liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
28. Add 100 µl of Solution PE to the center of the white filter membrane.
29. Incubate for 1 min at room temperature. Centrifuge at 15,000 x *g* for 1 minute.
30. Discard the MB Spin Column. The sample is now ready for downstream applications.
    Note: For 2D SDS-PAGE and in-solution proteolytic digestion for mass spectrometry, removal of the 1% SDS may be required. Refer to the Troubleshooting Guide for more information.

Protocol: Detailed

Important points before starting

- Add 100% ethanol (user provided) to IW Solution and WP Solution as indicated on the bottle label.
- Add 100% isopropanol (user provided) to RW Solution as indicated on the bottle label.
- To prepare a working stock of Solution HC, add 3.5 µl of β-mercaptoethanol (β-ME) per 350 µl of Solution HC. Alternatively, dithiothreitol (DTT) may be added to Solution HC to a final concentration of 1–10 mM. Use a fume hood when using β-ME or DTT.
- We strongly recommend using Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific cat. no. 78429). Use 3.5 µl of Halt Protease Inhibitor Cocktail per sample. Follow the manufacturer's recommendations when using other protease inhibitors.
- All protocol steps must be followed in the order written. DNA, RNA and protein subheadings are not stand alone protocols. Skipping steps will result in reduced binding efficiency and reduced recovery of the desired biomolecule.

Procedure

1. Add 1.8 ml of bacterial culture to a 2 ml Collection Tube (provided). Centrifuge for 3 min at 15,000 x *g*. Remove all of the supernatant with a pipette tip.
   Note: We recommend using no more than $1x10^9$ bacterial cells per sample. Centrifugation separates bacterial cells from the culture media. Lysis efficiency is significantly reduced when more than $1x10^9$ bacterial cells are used per sample
2. Re-suspend the cell pellet in 350 µl of Solution HC (working stock) by vortexing or pipetting. Transfer the re-suspended cells to a PowerBead Tube.
   Note: Solution HC must be freshly prepared with β-ME (or DTT) and protease inhibitors. Solution HC contains a chaotropic formulation that helps lyse and solubilize total bacterial nucleic acids and proteins. β-ME (or DTT) is a strong reducing agent that cleaves disulfide bonds. Protease inhibitors are required for many bacterial cultures that

produce an abundance of proteases. These compounds act together to maximize nucleic acid and protein stability and solubility.

3. Secure the PowerBead Tube horizontally using a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
   Note: Mechanical homogenization is performed with a PowerBead Tube with 0.1 mm glass beads to optimize lysis of both gram-positive and gram-negative bacterial cells.
4. Quick-spin the PowerBead Tube. Remove the cap and add 175 µl of Solution MR directly to the PowerBead Tube. Recap and vortex on high for at least 10 s to mix.
   Note: The addition of Solution MR directly to the PowerBead Tube solubilizes a small protein fraction that would otherwise be left behind.
5. Centrifuge the PowerBead Tube at 15,000 x *g* for 2 min at room temperature.
   Note: Centrifugation is used to clear the lysate of any remaining particulates or beads before the next step while leaving nucleic acids and proteins in solution.

DNA Purification

6. Transfer 350 µl of lysate from the PowerBead Tube directly to an MB Spin Column. Centrifuge for 1 min at 15,000 x *g*. Save flow-through for RNA purification (step 14).
   Note: It is normal to transfer some glass beads with the lysate.
   Note: DNA is selectively bound to the silica membrane in the MB Spin Column while the RNA and protein remain in the flow-through.
7. Transfer the MB Spin Column to a clean 2 ml Collection Tube (provided).
8. Add 650 µl of Solution EA and centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
   Note: Solution EA is an alcohol-based wash that is designed to remove residual salt while allowing DNA to remain bound to the MB Spin Column silica membrane.
9. Add 650 µl of IW Solution and centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
   Note: IW Solution ensures complete removal of Solution EA from the MB Spin Column filter membrane, which results in higher DNA yield and purity.
10. Centrifuge at 15,000 x *g* for 2 min. Being careful not to splash liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).

Note: This step removes residual IW Solution to prevent any carryover that could interfere with downstream DNA applications.

11. Add 100 µl of Solution EB to the center of the white filter membrane.
    Note: Adding Solution EB to the center of the filter membrane will ensure that the entire membrane is wet and DNA is eluted efficiently.
12. Incubate for 1 min at room temperature. Centrifuge at 15,000 x *g* for 1 min.
13. Discard the MB Spin Column. The DNA is now ready for downstream applications.

RNA purification

14. Add 350 µl of Solution RB to the flow-through from step 6. Vortex briefly on high.
15. Add the lysate to a new MB Spin Column and centrifuge at 15,000 x *g* for 1 min. Save the flow-through for protein purification (step 23).
    Note: RNA is selectively bound to the silica membrane in the MB Spin Column while proteins remain in the flow-through.
16. Transfer the MB Spin Column to a clean 2 ml Collection Tube (provided).
17. Add 650 µl of RW Solution. Centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
    Note: RW Solution is an alcohol-based wash that is designed to remove residual salt while allowing RNA to remain bound to the MB Spin Column silica membrane.
18. Add 650 µl of 100% ethanol (user provided). Centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.
    Note: 100% ethanol ensures complete removal of RW Solution from the MB Spin Column filter membrane, which results in higher RNA yield and purity.
19. Centrifuge at 15,000 x *g* for 2 min. Being careful not to splash liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
    Note: This step removes residual ethanol to prevent any carryover that could interfere with downstream RNA applications.
20. Add 100 µl of RNase-Free Water (provided) to the center of the white filter membrane.
    Note: Adding RNase-Free Water to the center of the filter membrane will ensure that the entire membrane is wet and RNA is eluted efficiently.

21. Incubate for 1 min at room temperature. Centrifuge at 15,000 x *g* for 1 min.
22. Discard the MB Spin Column. The RNA is now ready for downstream applications.

Protein purification

23. Add 650 µl of Solution AB to the flow-through from step 15. Vortex briefly on high.
24. Load up to 650 µl into a new MB Spin Column and centrifuge at 15,000 x *g* for 1 min.
25. Discard the flow-through and load the remaining sample volume into the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min and discard the flow-through.
    Note: Proteins are selectively bound to the silica membrane in the MB Spin Column while salts and impurities remain in the flow-through.
26. Add 650 µl of WP Solution. Centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
    Note: WP Solution is an alcohol-based wash that removes residual salts and impurities while allowing proteins to remain bound to the MB Spin Column.
27. Centrifuge at 15,000 x *g* for 2 min. Being careful not to splash liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
    Note: This step removes residual WP Solution to prevent any carryover that could interfere with protein elution and downstream applications.
28. Add 100 µl of Solution PE to the center of the white filter membrane.
    Note: Solution PE contains 1% SDS, which is used to elute membrane-bound proteins. HEPES stabilizes the elution solution at pH 8.0 and does not interfere with downstream applications, such as the BCA assay used to determine protein concentration.
    Note: Depending on the downstream application, alternate elution buffers, such as urea/thiourea and cleavable detergents, may be used. Use of these alternate elution buffers may result in reduced protein recovery. See the Troubleshooting Guide for additional information.
29. Incubate for 1 min at room temperature. Centrifuge at 15,000 x *g* for 1 minute.
30. Discard the MB Spin Column. The sample is now ready for downstream applications.
    Note: For 2D SDS-PAGE and in-solution proteolytic digestion for mass spectrometry, removal of the 1% SDS may be required. Refer to the Troubleshooting Guide for more information.

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
a) Low nucleic acid or protein yields	Several factors may affect nucleic acid and protein yields: A) Frozen bacterial stocks can degrade over long term storage and grow poorly or not at all. Poorly growing stocks will results in low nucleic acid and protein yields. B) Use fresh cultures. Extraction from older cultures may result in degraded material and lower yields. C) Nucleic acid and protein isolation must proceed in the order given in the protocol. All the buffers build on each other to optimally bind and elute the next biomolecule. Skipping steps can result in reduced yields.
b) Nucleic acids are degraded	Degraded nucleic acids are a common sign of nuclease activity. The older a bacterial culture is, the more potential to recover both degraded DNA and RNA. To reduce DNA degradation, use fresh overnight microbial cultures. To maintain RNA integrity during isolation and purification, add an RNase inhibitor such as β-ME or DTT to Solution HC as described in the Important points before starting sections on pages 9 and 12.
c) Proteins are degraded	Degraded proteins are a common sign of protease activity. The level of protease activity depends on sample type and may vary significantly from one cultured organism to another. Add protease inhibitors to Solution HC prior to bead beating as described in the Important points before starting sections on pages 9 and 12. Some cultures may require the addition of EDTA or other protease inhibitors.
d) Storing nucleic acids	DNA is eluted in 10 mM Tris, pH 8.0, while RNA is eluted in RNase-Free Water. Store both DNA and RNA at –15 to –30°C to prevent degradation or at –65 to –90°C for long-term storage.

	Comments and suggestions
e) Storing proteins	Proteins are eluted in 1% SDS/10 mM HEPES. For short term storage, store protein sample at 2–8°C. For long term storage, store protein sample at –15 to –30°C. The SDS present in the elution buffer will precipitate out of solution when stored at cold temperatures. Allow samples to thaw at room temperature. Briefly heat the protein sample at 55°C to re-dissolve the SDS prior to downstream applications.
f) Removing SDS from eluted proteins	Solution PE contains 1% SDS, which is required for maximum protein elution and recovery from the MB Spin Column. For some downstream applications (i.e., 2D SDS-PAGE or in-solution proteolytic digestion), SDS will need to be removed. To keep proteins in solution, a buffer exchange into 8 M Urea or guanidine thiocyanate can be done using traditional dialysis, a centrifugal filter device with a low molecular weight cut-off (e.g. 5–30 kDa) or a desalting column.
g) Alternate protein elution buffers	Alternatively, MB Spin Column-bound proteins can be eluted in a freshly prepared 7 M urea/2 M thiourea ± 4% CHAPS solution with minimal protein loss. Cleavable detergents, such as *Rapi*Gest™ SF Surfactant (Waters), have also been shown to be effective elution buffer alternatives but with reduced protein recovery. We have found that a ≥ 2% solution of *Rapi*Gest SF in Tris, pH 8.0 can serve as an alternative to Solution PE.

Ordering Information

Product	Contents	Cat. no.
AllPrep Bacterial DNA/RNA/Protein Kit (50)	For 50 preps: Extraction of protein and nucleic acids from bacterial cell cultures	47054
Related products		
AllPrep Fungal DNA/RNA/Protein Kit (50)	For 50 preps: Extraction of protein and nucleic acids from fungal cell cultures	47154
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
NoviPure® Microbial Protein Kit (50)	For 50 preps: Protein extraction from microbial cell cultures	47044
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 Bacterial DNA/RNA/Protein Kit

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