MO BIO Laboratories, Inc.

Saving You Time For Life

AllPrep® Bacterial DNA/RNA/Protein Kit (50)

Catalog No. 47054

Quantity: 50 preps

INSTRUCTION MANUAL

Version 09142016

Please recycle

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KIT CONTENTS

Component	Catalog #	Amount
AllPrep® Bacterial Bead Tubes	47054-BT	50
HC Solution	47054-1	20 ml
MR Solution	47054-2	10 ml
EA Solution	47054-3	36 ml
CB Solution	47054-4	18 ml
EB Solution	47054-5	6 ml
RB Solution	47054-6	20 ml
RW Solution	47054-7	18 ml
RNase-Free Water	47054-8	6 ml
AB Solution	47054-9	36 ml
WP Solution	47054-10	12 ml
PE Solution	47054-11	6 ml
Spin Filters	47054-SF	150
2 ml Collection Tubes	47054-T	300

KIT STORAGE

We recommend storing spin filters at 4°C. All other kit reagents and components can 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 Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All SDS 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.

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

EQUIPMENT REQUIRED

- ☐ Microcentrifuge (15,000 x *g*)
- ☐ Pipettors (1 µl - 1000 µl)
- ☐ Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
- ☐ Vortex Adapter for 1.5 - 2.0 ml tubes (MO BIO Catalog # 13000-V1-24)

REAGENTS & CONSUMABLES REQUIRED BUT NOT INCLUDED

- ☐ β-mercaptoethanol (β-me) or dithiothreitol (DTT)
- ☐ Halt™ Protease Inhibitor Cocktail (Thermo Scientific Catalog# 78429) or similar product
- ☐ 100% Ethanol
- ☐ 100% Isopropanol
- ☐ Pipette tips (volumes of 1 µl – 1000 µl)

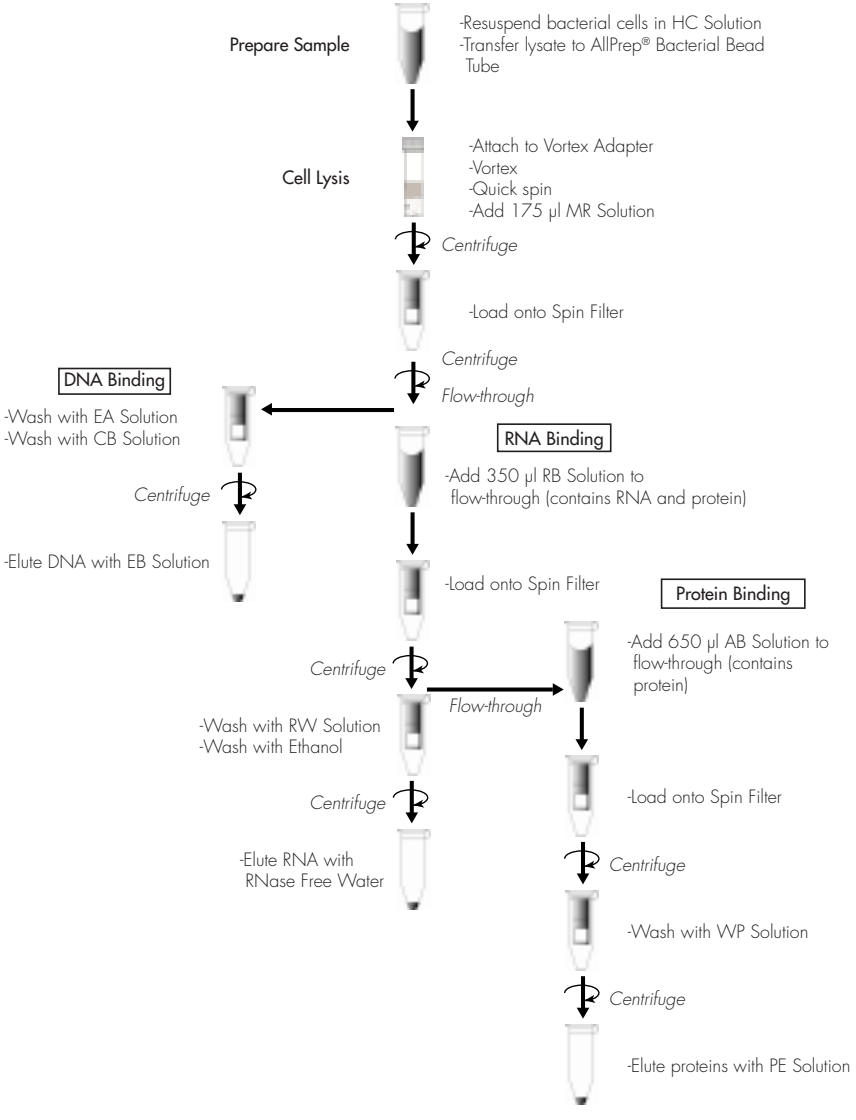
PROTOCOL OVERVIEW

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, small RNAs) are suitable for the most demanding downstream applications including PCR, qPCR, RT-PCR, and next-generation sequencing. 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.

This novel sequential isolation method begins with mechanical lysis of cultured bacterial cells using a 0.1 mm glass bead tube. 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 the 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.

AllPrep® Bacterial DNA/RNA/Protein Kit



A color version of this flowchart is available online at **www.mobio.com/47054/flowchart**

EXPERIENCED USER PROTOCOL
AllPrep® Bacterial DNA/RNA/Protein Kit
Catalog No. 47054

Please wear gloves at all times.

Important Notes Before Starting:

- **Prepare a Working Stock of HC Solution prior to each use. Use the table below as a guide.**
  - **Add β-me or DTT to HC Solution.**
    Add 3.5 µl of β-mercaptoethanol (β-me) per 350 µl of **HC Solution**. Alternatively, dithiothreitol (DTT) may be added to **HC Solution** to produce a final concentration of 1-10 mM. Use a fume hood when opening β-me or DTT to avoid exposure to the chemical.
  - **Add EDTA-free protease inhibitors to HC Solution.**

    MO BIO strongly recommends using Halt™ Protease Inhibitor Cocktail (Thermo Scientific™ Catalog#: 78429). Use 3.5 µl of Halt™ Protease Inhibitor Cocktail per sample. Follow manufacturer's recommendations when using other protease inhibitors.

Note

You can prepare larger amounts of **HC Solution** with fresh β-me and protease inhibitors according to the number of samples you need to process. DO NOT pre-mix and store **HC Solution** with β-me and protease inhibitors. *MIX AND USE FRESH.*

Recipe for Working Stock of HC Solution.		
Component	**1 sample**	**10 samples (example)**
HC Solution	350 µl	3.5 ml
ß-mercaptoethanol	3.5 µl	35 µl
Halt™ Protease Inhibitor Cocktail	3.5 µl	35 µl
Total Volume	357 µl	3.57 ml
Use 350 µl of freshly prepared Working Stock per sample.		

- **Prepare CB, RW, and WP wash solutions.**
  - Add 18 ml of 100% ethanol (user provided) to the **CB Solution** bottle. Mix well and put a check mark in the "ethanol added" box on the bottle cap label.

- Add 18 ml of 100% isopropanol (user provided) to the **RW Solution** bottle. Mix well and put a check mark on the bottle cap label to indicate the isopropanol has been added.

- Add 28 ml of 100% ethanol (user provided) to the **WP Solution** bottle. Mix well and put a check mark in the "ethanol added" box on the bottle cap label.

- **User provided, 100% ethanol is required as a second RNA wash in Step 19.**

- **Protein elution buffer contains 1% SDS in HEPES.**

  Some downstream applications may require the removal of SDS. See SDS Removal section in the "Hints and Troubleshooting Guide."

- **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.

1. Add up to 1.8 ml of bacterial culture to a 2 ml Collection Tube (provided) and centrifuge at 15,000 x *g* for 3 minutes at room temperature. Completely remove the media supernatant with a pipette tip.

Note

MO BIO recommends using no more than $1x10^9$ bacterial cells per sample.

2. Re-suspend the cell pellet in **350 µl of HC Solution (Working Stock)** by vortexing or pipetting. Transfer re-suspended cells to the **AllPrep® Bacterial Bead Tube**.

Note

HC Solution must be freshly prepared with β-me (or DTT) and protease inhibitors. Please see "Important Notes Before Starting" section for preparation instructions.

3. Secure the **AllPrep® Bacterial Bead Tube** horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1-24). Vortex at maximum speed for 10 minutes.

4. Quick spin the bead tube to remove residual lysate from the cap. Remove the bead tube cap and add **175 µl of MR Solution** directly to the bead tube. Recap the bead tube and vortex on high for at least 10 seconds to mix.

Note

Genomic DNA, total RNA, and total protein are all solubilized together but are then purified separately and sequentially.

5. Centrifuge the tubes at 15,000 x *g* for 2 minutes at room temperature.

Genomic DNA Purification

6. Transfer the lysate from the bead tube directly to a spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. **DO NOT discard the flow through. Save for Step 15 under Total RNA Purification**.

Note

It is normal to transfer some glass beads with the lysate.

7. Transfer the spin filter basket to a clean 2 ml Collection Tube (provided).

8. Add **650 µl of EA Solution** to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

9. **Confirm that 100% ethanol has been added to the CB Solution. Please see "Important Notes Before Starting" Section for preparation instructions.** Add **650 µl of CB Solution** to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

10. Centrifuge the empty spin filter at 15,000 x *g* for 2 minutes at room temperature to remove any residual solution.

11. Being careful not to splash liquid on the spin filter basket, place the spin filter in a new 2 ml Collection Tube (provided).

12. Add **100 µl of EB Solution** to the center of the white filter membrane. Incubate for a minimum of 1 minute at room temperature.

13. Centrifuge at 15,000 x *g* for 1 minute at room temperature.

14. Discard the spin filter. Your purified genomic DNA is now ready for downstream applications.

Total RNA Purification

15. Add **350 µl of RB Solution** to the flow through from Step 6. Vortex briefly on high to mix.

16. Add the lysate to a new spin filter (provided) and centrifuge at 15,000 x *g* for 1 minute at room temperature. **DO NOT discard the flow through. Save for Step 25 under Total Protein Purification.**

17. Transfer the spin filter basket to a clean 2 ml Collection Tube (provided).

18. **Confirm that 100% isopropanol has been added to the RW Solution. Please see "Important Notes Before Starting" Section for preparation instructions.** Add **650 µl of RW Solution** to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

19. Add **650 µl of 100% ethanol** (user provided) to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

20. Centrifuge the empty spin filter at 15,000 x *g* for 2 minutes at room temperature to remove any residual solution.

21. Being careful not to splash liquid on the spin filter basket, place the spin filter in a new 2 ml Collection Tube (provided).

22. Add **100 µl of RNase-Free Water** to the center of the white filter membrane. Incubate for a minimum of 1 minute at room temperature.

23. Centrifuge at 15,000 x *g* for 1 minute at room temperature.

24. Discard the spin filter. Your purified total RNA is now ready for downstream applications.

Total Protein Purification

25. Add **650 µl of AB Solution** to the flow through from Step 16. Vortex briefly on high to mix.

26. Load up to 650 µl onto a new spin filter (provided) and centrifuge at 15,000 x *g* for 1 minute at room temperature.

27. Discard the flow through and load the remaining sample volume onto the spin filter. Centrifuge at 15,000 x *g* for 1 minute at room temperature and discard the flow through.

28. **Confirm that 100% ethanol has been added to the WP Solution. Please see "Important Notes Before Starting" Section for preparation instructions.** Add **650 µl of WP Solution** to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

29. Centrifuge the empty spin filter at 15,000 x *g* for 2 minutes at room temperature to remove any residual solution.

30. Being careful not to splash liquid on the spin filter basket, place the spin filter in a new 2 ml Collection Tube (provided).

31. Add **100 µl of PE Solution** to the center of the white filter membrane. Incubate

Note

PE Solution contains 1% SDS which may need to be removed before some downstream applications. See SDS Removal section in the "Hints and Troubleshooting Guide."

Depending on the downstream application, alternate elution buffers may be used, such as urea/thiourea and cleavable detergents. Use of these alternate elution buffers may result in reduced protein recovery. See the "Hints and Troubleshooting Guide" for additional information.

for a minimum of 1 minute at room temperature.

32. Centrifuge at 15,000 x *g* for 1 minute at room temperature.

33. Discard the spin filter. Your sample is now ready for downstream applications. For 2D SDS-PAGE and in-solution proteolytic digestion for mass spectrometry, removal of the 1% SDS may be required. Please see the "Hints and Troubleshooting Guide" for additional information.

Thank you for choosing the AllPrep® Bacterial DNA/RNA/Protein Kit!

DETAILED PROTOCOL *(DESCRIBES WHAT IS HAPPENING AT EACH STEP)*
AllPrep® Bacterial DNA/RNA/Protein Kit
Catalog No. 47054

Please wear gloves at all times.

Important Notes Before Starting:

- **Prepare a Working Stock of HC Solution prior to each use. Use the table below as a guide.**
  - **Add β-me or DTT to HC Solution.**

    Add 3.5 µl of β-mercaptoethanol (β-me) per 350 µl of **HC Solution**. Alternatively, dithiothreitol (DTT) may be added to **HC Solution** to produce a final concentration of 1-10 mM. Use a fume hood when opening β-me or DTT to avoid exposure to the chemical.
  - **Add EDTA-free protease inhibitors to HC Solution.**

    MO BIO strongly recommends using Halt™ Protease Inhibitor Cocktail (Thermo Scientific Catalog#: 78429). Use 3.5 µl of Halt™ Protease Inhibitor Cocktail per sample. Follow manufacturer's recommendations when using other protease inhibitors.

Note

You can prepare larger amounts of **HC Solution** with fresh β-me and protease inhibitors according to the number of samples you need to process. DO NOT pre-mix and store **HC Solution** with β-me and protease inhibitors. *MIX AND USE FRESH.*

Recipe for Working Stock of HC Solution.		
Component	**1 sample**	**10 samples (example)**
HC Solution	350 µl	3.5 ml
ß-mercaptoethanol	3.5 µl	35 µl
Halt™ Protease Inhibitor Cocktail	3.5 µl	35 µl
Total Volume	357 µl	3.57 ml
Use 350 µl of freshly prepared Working Stock per sample.		

- **Prepare CB, RW, and WP wash solutions.**
  - Add 18 ml of 100% ethanol (user provided) to the **CB Solution** bottle. Mix well and put a check mark in the "ethanol added" box on the bottle cap label.

- Add 18 ml of 100% isopropanol (user provided) to the **RW Solution** bottle. Mix well and put a check mark on the bottle cap label to indicate the isopropanol has been added.

- Add 28 ml of 100% ethanol (user provided) to the **WP Solution** bottle. Mix well and put a check mark in the "ethanol added" box on the bottle cap label.

- **User provided, 100% ethanol is required as a second RNA wash in Step 19.**

- **Protein elution buffer contains 1% SDS in HEPES.**

  Some downstream applications may require the removal of SDS. See SDS Removal section in the "Hints and Troubleshooting Guide."

- **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.

1. Add up to 1.8 ml of bacterial culture to a 2 ml Collection Tube (provided) and centrifuge at 15,000 x *g* for 3 minutes at room temperature. Completely remove the media supernatant with a pipette tip.

Note

MO BIO recommends using no more than $1x10^9$ bacterial cells per sample.

What is happening: 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 HC Solution (Working Stock)** by vortexing or pipetting. Transfer re-suspended cells to the **AllPrep® Bacterial Bead Tube**.

Note

HC Solution must be freshly prepared with β-me (or DTT) and protease inhibitors. Please see "Important Notes Before Starting" section for preparation instructions.

What is happening: HC Solution 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 **AllPrep® Bacterial Bead Tube** horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1-24). Vortex at maximum speed for 10 minutes.

   What is happening: Mechanical homogenization is performed with a 0.1 mm glass bead tube optimized to lyse both gram-positive and gram-negative bacterial cells.

4. Quick spin the bead tube to remove residual lysate from the cap. Remove the bead tube cap and add **175 µl of MR Solution** directly to the bead tube. Recap the bead tube and vortex on high for at least 10 seconds to mix.

   Note

   Genomic DNA, total RNA, and total protein are all solubilized together and will be purified separately and sequentially.

   What is happening: The addition of MR Solution directly to the bead tube solubilizes a small protein fraction that would otherwise be left behind.

5. Centrifuge the tubes at 15,000 x *g* for 2 minutes at room temperature.

   What is happening: Centrifugation is used to clear the lysate of any remaining particulates and beads before the next step while leaving nucleic acids and proteins in solution.

Genomic DNA Purification

6. Transfer the lysate from the bead tube directly to a spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. **DO NOT discard the flow through. Save for Step 15 under Total RNA Purification.**

   Note

   It is normal to transfer some glass beads with the lysate.

   What is happening: DNA is selectively bound to the silica membrane in the spin filter basket while the RNA and protein remain in the flow through.

7. Transfer the spin filter basket to a clean 2 ml Collection Tube (provided).

8. Add **650 µl of EA Solution** to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

   What is happening: EA Solution is an alcohol based wash that is designed to remove residual salt while allowing the DNA to remain bound to the silica membrane.

9. **Confirm that 100% ethanol has been added to the CB Solution. Please see "Important Notes Before Starting" Section for preparation instructions.** Add **650 µl of CB Solution** to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

What is happening: CB Solution ensures complete removal of EA Solution which results in higher DNA yield and purity.

10. Centrifuge the empty spin filter at 15,000 x *g* for 2 minutes at room temperature to remove any residual solution.

What is happening: Residual CB solution is removed which prevents solution carryover that could interfere with downstream DNA applications.

11. Being careful not to splash liquid on the spin filter basket, place the spin filter in a new 2 ml Collection Tube (provided).

12. Add **100 µl of EB Solution** to the center of the white filter membrane. Incubate for a minimum of 1 minute at room temperature.

What is happening: Placing the EB solution in the center of the filter membrane will ensure that the entire membrane is wetted and DNA is efficiently eluted.

13. Centrifuge at 15,000 x *g* for 1 minute at room temperature.

14. Discard the spin filter. Your purified genomic DNA is now ready for downstream applications.

Total RNA Purification

15. Add **350 µl of RB Solution** to the flow through from Step 6. Vortex briefly on high to mix.

What is happening: RB solution is an alcohol based, RNA selective bind, added to the flow through containing RNA and proteins.

16. Add the lysate to a new spin filter (provided) and centrifuge at 15,000 x *g* for 1 minute at room temperature. **DO NOT discard the flow through. Save for Step 25 under Total Protein Purification.**

What is happening: RNA is selectively bound to the silica membrane in the spin filter basket while proteins remain in the flow through.

17. Transfer the spin filter basket to a clean 2 ml Collection Tube (provided).

18. **Confirm that 100% isopropanol has been added to the RW Solution. Please see "Important Notes Before Starting" Section for preparation instructions.** Add **650 µl of RW Solution** to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

What is happening: RW Solution is an alcohol based wash that is designed to remove residual salt while allowing the RNA to remain bound to the silica membrane.

19. Add **650 µl of 100% ethanol (user provided)** to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

What is happening: 100% ethanol ensures complete removal of RW Solution which results in higher RNA yield and purity.

20. Centrifuge the empty spin filter at 15,000 x *g* for 2 minutes at room temperature to remove any residual solution.

What is happening: Residual ethanol solution is removed which prevents solution carryover that could interfere with downstream RNA applications.

21. Being careful not to splash liquid on the spin filter basket, place the spin filter in a new 2 ml Collection Tube (provided).

22. Add **100 µl of RNase-Free Water** to the center of the white filter membrane. Incubate for a minimum of 1 minute at room temperature.

What is happening: Placing the RNase-Free Water in the center of the filter membrane will ensure that the entire membrane is wetted and RNA is efficiently eluted.

23. Centrifuge at 15,000 x *g* for 1 minute at room temperature.

24. Discard the spin filter. Your purified total RNA is now ready for downstream applications.

Total Protein Purification

25. Add **650 µl of AB Solution** to the flow through from Step 16. Vortex briefly on high to mix.

What is happening: AB Solution is a novel bind, added to the flow through that now contains only denatured proteins.

26. Load up to 650 µl onto a new spin filter (provided) and centrifuge at 15,000 x *g* for 1 minute at room temperature.

27. Discard the flow through and load the remaining sample volume onto the spin filter. Centrifuge at 15,000 x *g* for 1 minute at room temperature and discard the flow through.

What is happening: Proteins are selectively bound to the silica membrane in the spin filter basket while salts and impurities remain in the flow through.

28. Confirm that 100% ethanol has been added to the WP Solution. Please see "Important Notes Before Starting" Section for preparation instructions. Add **650 µl of WP Solution** to the spin filter and centrifuge at 15,000 x *g* for 1 minute at room temperature. Discard the flow through.

What is happening: WP Solution is an alcohol based wash that removes residual salts and impurities while allowing proteins to remain bound to the silica spin column. Salt carryover will cause SDS precipitation in the final elution.

29. Centrifuge the empty spin filter at 15,000 x *g* for 2 minutes at room temperature to remove any residual solution.

What is happening: Residual WP Solution is removed which prevents solution carryover that may interfere with protein elution and downstream applications.

30. Being careful not to splash liquid on the spin filter basket, place the spin filter in a new 2 ml Collection Tube (provided).

31. Add **100 µl of PE Solution** to the center of the white filter membrane. Incubate for a minimum of 1 minute at room temperature.

Note

PE Solution contains 1% SDS which may need to be removed before some downstream applications. See SDS Removal section in the "Hints and Troubleshooting Guide".

Depending on the downstream application, alternate elution buffers may be used, such as urea/thiourea and cleavable detergents. Use of these alternate elution buffers may result in reduced protein recovery. See the "Hints and Troubleshooting Guide" for additional information.

What is happening: 1% SDS is used to elute membrane-bound proteins. HEPES stabilizes the elution solution at pH 8 and does not interfere with downstream applications such as the BCA assay used to determine protein concentration.

32. Centrifuge at 15,000 x *g* for 1 minute at room temperature.

33. Discard the spin filter. Your sample is now ready for downstream applications. For 2D SDS-PAGE and in-solution proteolytic digestion for mass spectrometry, removal of the 1% SDS may be required. Please see the "Hints and Troubleshooting Guide" for additional information.

Thank you for choosing the AllPrep® Bacterial DNA/RNA/Protein Kit!

HINTS AND TROUBLESHOOTING GUIDE

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.

Degraded Nucleic Acids
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 a RNase inhibitor such as β-me or DTT to **HC Solution** as described on page 7 under "Important Notes Before Starting."

Degraded Proteins
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 HC Solution prior to bead beating according to the preparation instructions found in the "Important Notes Before Starting" section. Some cultures may require the addition of EDTA or other protease inhibitors.

Nucleic Acid Storage
DNA is eluted in 10 mM Tris, pH 8.0 while RNA is eluted in RNase-Free Water. Store both DNA and RNA at -20°C to prevent degradation or at -80°C for long term storage.

Protein Storage
Proteins are eluted in 1% SDS/10 mM HEPES. For short term storage, store your protein sample at 4°C. For long term storage, store your protein sample at -20°C. The SDS present in the elution buffer will precipitate out of solution when stored at cold temperatures. Allow sample to thaw at room temperature. Briefly heat your protein sample at 55°C to redissolve the SDS prior to downstream applications.

SDS Removal From Protein Elution Solution
The protein elution solution (PE Solution) contains 1% SDS which is required for maximum protein elution and recovery from the spin filter. For some downstream applications (i.e. 2D SDS-PAGE, in-solution proteolytic digestion), SDS will need to be removed. To keep proteins in solution, a buffer exchange into 8M 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.

Alternate Protein Elution Buffers

Silica bound proteins can alternatively be eluted in a freshly prepared 7M urea/2M thiourea ± 4% CHAPS solution with minimal protein loss. Cleavable detergents, such as RapiGest 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 RapiGest in Tris, pH 8.0 can serve as an alternative to elution with PE Solution.

PRODUCTS RECOMMENDED FOR YOU

Product	Catalog #	Quantity
NoviPure® Microbial Protein Kit (50)	47044	50 preps
AllPrep® Fungal DNA/RNA/Protein Kit (50)	47154	50 preps
NoviPure® Soil Protein Extraction Kit	30000-20	20 preps

TECHNICAL SUPPORT

Phone: Toll Free 800-606-6246, or 760-929-9911

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Mail: MO BIO Laboratories, Inc., 2746 Loker Ave West, Carlsbad, CA 92010

Committed to resolving your technical questions promptly, our technical support team is trained to work with you to rapidly and effectively trouble shoots any issues. We commit to providing you with relevant online support resources that help you complete your research projects.

Frequently Asked Questions:
https://mobio.com/faq

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