MO BIO Laboratories, Inc.

Saving You Time For Life

NoviPure® Microbial Protein Kit (50)

Catalog No. 47044

Quantity: 50 preps

INSTRUCTION MANUAL

Version 04202016

Please recycle

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KIT CONTENTS

Component	Catalog #	Amount
NoviPure® Microbial Bead Tubes	47044-BT	50
PL Solution	47044-1	22 ml
VN Solution	47044-2	25 ml
AB Solution	47044-3	66 ml
RNase-Free Water	47044-4	72 ml
PE Solution	47044-5	6 ml
Antifoam	47044-6	100 µl
Spin Filters	47044-SF	50
2 ml Collection Tubes	47044-T	250

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 contact or ingestion. 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
- ☐ Pipette tips (volumes of 1 µl – 1000 µl)

PROTOCOL OVERVIEW

The NoviPure® Microbial Protein Kit is designed to isolate total cellular proteins from microbial cultures in a user-friendly spin filter format. Bead beating in the presence of a strong chaotropic agent enables efficient lysis and solubilization of total proteins from a large diversity of microbial species including fungi, gram-negative and gram-positive bacteria. The use of silica spin filters to achieve reversible immobilization of proteins—a patent-pending technical advance in the chemistry of protein extraction—greatly simplifies the protein isolation process by removing cumbersome and bias-inducing protein precipitation steps. Following in-gel trypsin digestion, isolated proteins are suitable for downstream applications such as 1D SDS-PAGE and mass spectroscopy. Some applications, such as 2D SDS-PAGE and in-solution proteolytic digestion for mass spectrometry may require an additional detergent removal step following protein elution.

This novel protein isolation method begins with the mechanical lysis of cultured microbial cells using an optimized mixture of glass beads. Proteins released during homogenization are denatured and solubilized by a strong chaotropic agent. An optimized two-step binding process is then used to immobilize all solubilized proteins onto a silica spin filter. In the first step, a salt solution is added to the microbial lysate and passed over a silica spin filter to bind most proteins. In the second step, the flow through is adjusted with a second salt buffer and passed through the same spin filter. This second salt buffer promotes binding of low molecular weight proteins to the silica spin filter. Immobilized proteins are then washed and eluted.

NoviPure® Microbial Protein Kit

Prepare Sample

-Resuspend bacterial cells in PL Solution

Cell Lysis

-Transfer cells to NoviPure® Microbial Bead Tube
-Attach to vortex adapter
-Vortex

Centrifuge

-Add VN Solution

Bind Proteins

-Load onto Spin Filter

Centrifuge

-Add AB Solution to **flow-through**

-Load onto **same** Spin Filter

Centrifuge

Wash

-Wash with RNase-Free Water

Centrifuge

Elute

-Elute with PE Solution

EXPERIENCED USER PROTOCOL

NoviPure® Microbial Protein Kit

Catalog No. 47044

Please wear gloves at all times

Important Notes Before Starting

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

    Add 4 µl of β-me per 400 µl of **PL Solution**. Alternatively, dithiothreitol (DTT) may be added to **PL Solution** to 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 PL Solution.**

    MO BIO strongly recommends using Halt™ Protease Inhibitor Cocktail (Thermo Scientific™ Catalog #78429). Use 4 µl of Halt™ Protease Inhibitor Cocktail per 400 µl of **PL Solution**. Follow manufacturer's recommendations when using other protease inhibitors.

  - **Add antifoam (provided) to PL Solution.**

    Certain microbial cultures such as *E. coli* produce an excessive amount of foam during bead beating. Foam production causes inconsistent lysis and impacts final protein yields. Add 0.4 µl of antifoam per sample to a final concentration of 0.1%. We recommend the addition of antifoam regardless of microbial species. Vortex antifoam prior to use.

Note

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

Recipe for Working Stock of PL Solution.		
Component	**1 sample**	**10 samples (example)**
PL Solution	400 µl	4 ml
ß-mercaptoethanol	4 µl	40 µl
Halt™ Protease Inhibitor Cocktail	4 µl	40 µl
Antifoam	0.4 µl	4 µl
Total Volume	408.4	4.084 ml
Use 400 µl of freshly prepared Working Stock per sample.		

- **PE Solution (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."

- **Removal of nucleic acids.**

  This protocol will result in the co-isolation of partially degraded DNA and RNA. To remove nucleic acids, follow the on-column nuclease digestion protocol provided in the "Hints and Troubleshooting Guide."

1. Add up to 1.8 ml of microbial (bacteria, yeast) 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 $1x10^8$ fungal cells or $1x10^9$ bacterial cells per sample.

2. Re-suspend the cell pellet in **400 µl of PL Solution (Working Stock)** by vortexing or pipetting. Transfer re-suspended cells to the **NoviPure® Microbial Bead Tube**.

Note

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

3. Secure the **NoviPure® Microbial Bead Tube** horizontally on the MO BIO Vortex Adapter (MO BIO Catalog# 13000-V1-24). Vortex at maximum speed for 10 minutes.

4. Centrifuge the tubes at 15,000 x g for 1 minute at room temperature.

5. Transfer the lysate to a clean 2 ml Collection Tube (provided).

Note

Expect 200 to 300 µl of lysate. It is normal to transfer some glass beads with the lysate.

6. Add **450 µl of VN Solution** to the lysate. Vortex briefly on high to mix.

Note

The sample may become opaque upon the addition of **VN Solution**. This is normal.

7. Load up to 700 µl onto a spin filter. Centrifuge at 15,000 x g for 1 minute at room temperature. **DO NOT discard the flow through**.
8. Transfer the spin filter basket to a clean 2 ml Collection Tube (provided).
9. Add **600 µl of AB Solution** to the **flow through** from step 7. Vortex briefly on high to mix.
10. Load 650 µl onto the **same** spin filter. Centrifuge at 15,000 x g for 1 minute at room temperature. Discard 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.
11. Add **650 µl of RNase-Free Water** to the spin filter. Centrifuge at 15,000 x g for 1 minute at room temperature. Discard the flow through.
12. Centrifuge the empty spin filter at 15,000 x g for 2 minutes at room temperature to remove any residual solution.
13. Being careful not to splash liquid on the spin filter basket, place the spin filter in a new 2 ml Collection Tube (provided).

Note

If desired, on-column removal of nucleic acids may be carried out after this step using the protocol provided in the "Hints and Troubleshooting Guide."

14. Add **100 µl of PE Solution** to the center of the spin 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."

Note

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

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

16. Discard the spin filter. Your sample is now ready for downstream applications such as 1D SDS-PAGE. For 2D SDS-PAGE and in-solution proteolytic digestion for mass spectrometry, removal of the 1% SDS may be required. For long-term storage we recommend storing your samples at -20°C. Please see the "Hints and Troubleshooting Guide" for additional information.

Thank you for choosing the NoviPure® Microbial Protein Kit!

DETAILED PROTOCOL *(DESCRIBES WHAT IS HAPPENING AT EACH STEP)*

NoviPure® Microbial Protein Kit

Catalog No. 47044

Please wear gloves at all times

Important Notes Before Starting

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

    Add 4 µl of β-me per 400 µl of **PL Solution**. Alternatively, dithiothreitol (DTT) may be added to **PL Solution** to 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 PL Solution.**

    MO BIO strongly recommends using Halt™ Protease Inhibitor Cocktail (Thermo Scientific™ Catalog #78429). Use 4 µl of Halt™ Protease Inhibitor Cocktail per 400 µl of **PL Solution**. Follow manufacturer's recommendations when using other protease inhibitors.

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    Certain microbial cultures such as E. coli produce an excessive amount of foam during bead beating. Foam production causes inconsistent lysis and impacts final protein yields. Add 0.4 µl of antifoam per sample to a final concentration of 0.1%. We recommend the addition of antifoam regardless of microbial species. Vortex antifoam prior to use.

Note

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

Recipe for Working Stock of PL Solution.		
Component	**1 sample**	**10 samples (example)**
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Total Volume	408.4	4.084 ml
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- **PE Solution (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."

- **Removal of nucleic acids.**

  This protocol will result in the co-isolation of partially degraded DNA and RNA. To remove nucleic acids, follow the on-column nuclease digestion protocol provided in the "Hints and Troubleshooting Guide."

1. Add up to 1.8 ml of microbial (bacteria, yeast) 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 $1x10^8$ fungal cells or $1x10^9$ bacterial cells per sample.

What is happening: Centrifugation separates microbial cells from the culture media. Lysis efficiency is significantly reduced when more than $1x10^8$ fungal cells or $1x10^9$ bacterial cells are used per sample. Addition of excess sample leads to cell debris carry-over and precipitation upon the addition of VN Solution and AB Solution.

2. Re-suspend the cell pellet in **400 µl of PL Solution (Working Stock)** by vortexing or pipetting. Transfer re-suspended cells to the **NoviPure® Microbial Bead Tube.**

Note

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

What is happening: PL Solution contains a strong chaotropic agent that helps lyse and solubilize total microbial proteins. ß-me (or DTT) is a strong reducing agent that cleaves disulfide bonds. Protease inhibitors are required for many microbial cultures that produce an abundance of proteases. These compounds act together to maximize protein stability and solubility.

3. Secure the **NoviPure® Microbial Bead Tube** horizontally on the MO BIO Vortex Adapter (MO BIO Catalog# 13000-V1-24). Vortex at maximum speed for 10 minutes.

What is happening: Mechanical homogenization is performed with a mixed glass bead tube optimized to lyse both fungal and bacterial cells.

4. Centrifuge the tubes at 15,000 x g for 1 minute 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 proteins in solution.

5. Transfer the lysate to a clean 2 ml Collection Tube (provided).

Note

Expect 200 to 300 µl of lysate. It is normal to transfer some glass beads with the lysate.

6. Add **450 µl of VN Solution** to the lysate. Vortex briefly on high to mix.

Note

The sample may become opaque upon the addition of **VN Solution**. This is normal.

What is happening: VN Solution is a neutral salt solution that drives the association of proteins with the silica membrane surface.

7. Load up to 700 µl onto a spin filter. Centrifuge at 15,000 x g for 1 minute at room temperature. **DO NOT discard the flow through.**

What is happening: Proteins bind to the silica spin filter in the presence of VN Solution.

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

9. Add **600 µl of AB Solution** to the **flow through** from step 7. Vortex briefly on high to mix.

What is happening: The flow through can contain low molecular weight proteins that require alternative binding conditions. The addition of AB Solution adjusts the binding conditions and promotes binding of low molecular weight proteins to the spin filter.

10. Load 650 µl onto the **same** spin filter. Centrifuge at 15,000 x g for 1 minute at room temperature. Discard 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: The remaining proteins bind to the silica spin filter without displacing previously bound proteins.

11. Add **650 µl of RNase-Free Water** to the spin filter. Centrifuge at 15,000 x g for 1 minute at room temperature. Discard the flow through.

What is happening: RNase-Free Water removes residual salts from the silica filter membrane. Salt carryover will cause SDS precipitation in the final elution.

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

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

Note

If desired, on-column removal of nucleic acids may be carried out after this step using the protocol provided in the "Hints and Troubleshooting Guide."

14. Add **100 µl of PE Solution** to the center of the spin 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."

Note

Depending on the downstream application, alternate elution buffers may be used, such as urea:thiourea or cleavable detergents. Use of these alternative 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 spin filter-bound proteins. HEPES stabilizes the elution solution at pH 8 and does not interfere with downstream applications such as the BCA assay used to quantify protein concentration.

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

16. Discard the spin filter. Your sample is now ready for downstream applications such as 1D SDS-PAGE. For 2D SDS-PAGE and in-solution proteolytic digestion for mass spectrometry, removal of the 1% SDS may be required. For long-term storage we recommend storing your samples at -20°C. Please see the "Hints and Troubleshooting Guide" for additional information.

Thank you for choosing the NoviPure® Microbial Protein Kit!

HINTS AND TROUBLESHOOTING GUIDE

Protein Precipitation
The NoviPure® Microbial Protein Kit is designed to process $1x10^8$ fungal cells or $1x10^9$ bacterial cells. This cell density typically equates to 1.8 – 2.0 ml of overnight culture. The addition of too much starting material decreases lysis efficiency. Intact cells, as well as cell debris, will carry over into the binding steps. This excess material leads to precipitation and may clog the spin filter. Decreasing the amount of starting material may limit/prevent precipitation and thereby increase protein yields.

The two-step binding system is designed to sequentially immobilize a diverse range of proteins onto the silica spin filter. Deviating from the protocol by combining **VN Solution** and **AB Solution** or adding these solutions out of order may result in irreversible protein precipitation.

Do not leave samples unattended for extended periods of time. We recommend processing samples until completion to avoid precipitation.

Inconsistent Yields
Inconsistent yields are most frequently a consequence of foam formation during bead beating. Foam acts as a cushion within the bead tube and negatively impacts lysis efficiency. Add the provided antifoam to **PL Solution** prior to bead beating according to the preparation instructions found in the "Important Notes Before Starting" section. We recommend the addition of antifoam to your sample regardless of microbial species.

Degraded Proteins
Degraded proteins are a common sign of protease activity. The level of protease activity is based on sample type and may vary significantly between microbial species. Add protease inhibitors to **PL Solution** prior to bead beating according to the preparation instructions found in the "Important Notes Before Starting" section. Processing samples on ice may decrease protease activity but is not a recommended alternative due to the possibility of protein precipitation.

Protein Storage
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 re-solubilize the SDS prior to downstream applications.

SDS removal
The elution buffer contains 1% SDS which is required for maximum protein 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, conduct a buffer exchange with 8M Urea, guanidine thiocyanate, or other suitable chaotrope/detergent solution using traditional dialysis tubing, a low molecular weight cut-off (e.g., < 5 kD) centrifugal filter device or a desalting column.

Alternate 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. A ≥ 2% solution of RapiGest™ in Tris, pH 8.0 can serve as an alternative elution buffer.

Nucleic Acid Removal
The NoviPure® Microbial Protein Kit co-isolates nucleic acids. These nucleic acids are associated with proteins and will elute into your final sample. Nucleic acid carryover may negatively impact some downstream applications such as 2D electrophoresis. Since the SDS present in **PE Solution** will inhibit nucleases, we recommend the following on-column nuclease digestion protocol if you need to remove nucleic acids from your sample. On-column treatment will permit you to enzymatically digest residual nucleic acids present in your protein sample prior to elution from the silica spin filter.

The DNase Max® Kit (MO BIO Catalog# 15200-50) and RNase A enzyme (25 mg/ml) can be used to complete the on-column nuclease digestion. Additional volumes of **AB Solution and RNase-Free Water** are required for this protocol and have been provided with the NoviPure® Microbial Protein Kit.

1. Prepare 50 µl of 1X DNase Max® Buffer by adding **5 µl of 10X DNase Max® Buffer** to **45 µl of RNase-Free Water** per sample.

2. Add **1 µl DNase Max® Enzyme** and **1 µl RNase A** to the 1X DNase Max® Buffer per sample.

3. Add 50 µl of the nuclease mix to the center of the spin filter membrane from step 13 of the NoviPure® Microbial Protein Kit protocol.

4. Incubate the reaction at room temperature for 15 minutes.

5. Add **600 µl of AB Solution** to the spin filter. Centrifuge at 15,000 x g for 1 minute at room temperature. **DO NOT discard the flow through.**

6. Load the entire flow through volume back onto the same spin filter. Centrifuge at 15,000 x g for 1 minute at room temperature. Discard the flow through.

7. Add **650 µl of RNase-Free Water** to the spin filter. Centrifuge at 15,000 x g for 1 minute at room temperature. Discard the flow through.

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

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

10. Proceed with step 14 of the NoviPure® Microbial Protein Kit protocol.

PRODUCTS RECOMMENDED FOR YOU

Product	Catalog #	Quantity
UltraClean® Microbial DNA Isolation Kit	12224-50 12224-250	50 preps 250 preps
PowerLyzer® UltraClean® Microbial DNA Isolation Kit	12255-50	50 preps
PowerMag® Microbial DNA Isolation Kit	27200-4	4 x 96 preps
UltraClean® -htp 96 Well Microbial DNA Isolation Kit	10196-4	4 x 96 preps
DNase Max® Kit	15200-50	50 preps

TECHNICAL SUPPORT

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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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