April 2018

NoviPure® Microbial Protein Kit Handbook

For the extraction of protein from microbial cell cultures

Sample to Insight

QIAGEN

Contents

Kit Contents

NoviPure Microbial Protein Kit	**(50)**
Catalog no.	**47044**
Number of preps	**50**
NoviPure Microbial Bead Tubes	50
MB Spin Columns	50
Solution AB	66 ml
Solution PE	6 ml
Solution PL	22 ml
Solution VN	25 ml
RNase-Free Water	100 ml
Antifoam	100 µl
Collection Tubes (2 ml)	5 x 50
Quick Start Protocol	1

Storage

All other components of the NoviPure Microbial Protein Kit can be stored at room temperature (15–25°C) until the expiration date printed on the box label.

Intended Use

All NoviPure 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 PL contains guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer 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 NoviPure Microbial Protein Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The NoviPure Microbial Protein Kit is designed to isolate total cellular proteins from microbial cultures in a user-friendly spin-column format.

Principle and procedure

The NoviPure Microbial Protein Kit uses bead beating in the presence of a strong chaotropic agent to efficiently lyse and solubilize total proteins from a large variety of microbial species including fungi, gram-negative and gram-positive bacteria. The use of silica spin filters (in the MB Spin Columns) 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 spectrometry. 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.

The NoviPure Microbial Protein Kit protocol starts 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 silica spin filters. First, a salt solution is added to the microbial lysate and passed through a silica spin filter that binds most proteins. Second, the flow-through is adjusted with a salt buffer and passed through the same silica spin filter again. The second salt buffer promotes binding of low molecular weight proteins to the silica spin filter. Immobilized proteins are then washed and eluted.

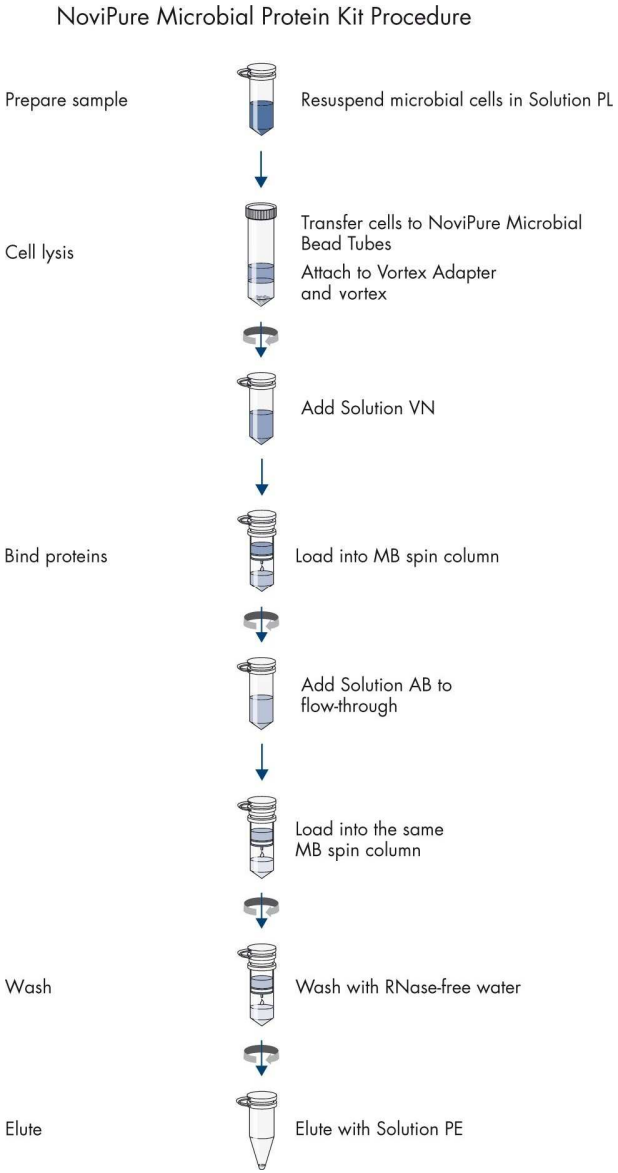

Figure 1. NoviPure Microbial Protein Kit procedure.

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 to 15,000 x *g*)
- Pipettors (1–1000 µl)
- Pipette tips (1–1000 µl)
- Vortex-Genie® 2 Vortex
- Vortex Adapter for vortexing 1.5–2 ml tubes (cat. no. 13000-V1-24)
- Halt™ Protease Inhibitor Cocktail (Thermo Fisher Scientific cat. no. 78429) or similar product
- β-Mercaptoethanol (β-ME) or dithiothreitol (DTT)

Protocol: Experienced User

Important points before starting

- Prepare a working stock of Solution PL prior to each use by adding 4 µl of β-mercaptoethanol (β-ME) per 400 µl of Solution PL. Alternatively, dithiothreitol (DTT) may be added to Solution PL to a final concentration of 1–10 mM. Use a fume hood when using β-ME or DTT.
- Add EDTA-free protease inhibitors to Solution PL. We recommend using Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific cat. no. 78429). Use 4 µl of Halt Protease Inhibitor Cocktail per 400 µl of Solution PL. Follow the manufacturer's recommendations when using other protease inhibitors.
- Solution PE (elution buffer) contains 1% SDS in HEPES. Some downstream applications may require the removal of SDS. Refer to the Troubleshooting Guide for additional information.
- This protocol will co-isolate partially degraded DNA and RNA. Refer to the Troubleshooting Guide for additional information about removing nucleic acids.
- Add 0.4 µl of Antifoam (provided) to each sample (400 µl) of Solution PL to a final concentration of 0.1%. Vortex Antifoam prior to use. Certain microbial cultures such as *E. coli* produce an excessive amount of foam during bead beating. Foam production can cause inconsistent lysis and impacts final protein yields. We recommend adding Antifoam regardless of microbial species.

Procedure

1. Add up to 1.8 ml of microbial (bacteria or yeast) culture to a 2 ml Collection Tube (provided) and centrifuge at 15,000 x *g* for 3 min at room temperature. Completely remove the media supernatant with a pipette tip.
   Note: We recommend using no more than $1x10^8$ fungal cells or $1x10^9$ bacterial cells per sample.

2. Resuspend the cell pellet in 400 µl of Solution PL (working stock) by vortexing or pipetting. Transfer resuspended cells to the NoviPure Microbial Bead Tube.
3. Secure the NoviPure Microbial Bead Tube horizontally on a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
4. Centrifuge the tubes at 15,000 x *g* for 1 min at room temperature.
5. Transfer the lysate to a clean 2 ml Collection Tube (provided).
   Note: Expect 200–300 µl. It is normal to transfer some glass beads with the lysate.
6. Add 450 µl of Solution VN to the lysate. Vortex briefly on high to mix.
   Note: The sample may become opaque upon the addition of Solution VN. This is normal.
7. Load up to 700 µl of the lysate/Solution VN mix into an MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. **Do not** discard the flow-through.
8. Transfer the MB Spin Column to a clean 2 ml Collection Tube (provided).
9. Add 600 µl of Solution AB to the flow-through from step 7. Vortex briefly on high to mix.
10. Load 650 µl of the mix from Step 9 into the same MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. Discard the flow-through and load the remaining sample volume into the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature and discard the flow-through.
11. Add 650 µl of RNase-Free Water to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. Discard the flow-through.
12. Centrifuge the empty MB Spin Column at 15,000 x *g* for 2 min at room temperature.
13. Being careful not to splash any liquid on the filter basket, place the MB Spin Column 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 Appendix A.
14. Add 100 µl of PE Solution to the center of the spin filter membrane. Incubate for a minimum of 1 min at room temperature.
    Note: Depending on the downstream application, alternate elution buffers, such as urea:thiourea or cleavable detergents, may be used. Using alternative elution buffers may

result in reduced protein recovery. Refer to the Troubleshooting Guide for additional information.

15. Centrifuge at 15,000 x *g* for 1 min at room temperature.
16. 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 1% SDS may be required. Refer to the Troubleshooting Guide for additional information.

Protocol: Detailed

Important points before starting

- Prepare a working stock of Solution PL prior to each use by adding 4 µl of β-mercaptoethanol (β-ME) per 400 µl of Solution PL. Alternatively, dithiothreitol (DTT) may be added to Solution PL to a final concentration of 1–10 mM. Use a fume hood when using β-ME or DTT.
- Add EDTA-free protease inhibitors to Solution PL. We recommend using Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific cat. no. 78429). Use 4 µl of Halt Protease Inhibitor Cocktail per 400 µl of Solution PL. Follow the manufacturer's recommendations when using other protease inhibitors.
- Solution PE (elution buffer) contains 1% SDS in HEPES. Some downstream applications may require the removal of SDS. Refer to the Troubleshooting Guide for additional information.
- This protocol will co-isolate partially degraded DNA and RNA. Refer to the Troubleshooting Guide for additional information about removing nucleic acids.
- Add 0.4 µl of Antifoam (provided) to each sample (400 µl) of Solution PL to a final concentration of 0.1%. Vortex Antifoam prior to use. Certain microbial cultures such as *E. coli* produce an excessive amount of foam during bead beating. Foam production can cause inconsistent lysis and impacts final protein yields. We recommend adding Antifoam regardless of microbial species.

Procedure

1. Add up to 1.8 ml of microbial (bacteria or yeast) culture to a 2 ml Collection Tube (provided) and centrifuge at 15,000 x *g* for 3 min at room temperature. Completely remove the media supernatant with a pipette tip.
   Note: We recommend using no more than $1x10^8$ fungal cells or $1x10^9$ bacterial cells per sample.

Note: 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 Solution VN and Solution AB.

2. Resuspend the cell pellet in 400 µl of Solution PL (working stock) by vortexing or pipetting. Transfer resuspended cells to the NoviPure Microbial Bead Tube.
   Note: Solution PL contains a strong chaotropic agent that helps lyse and solubilize total microbial proteins. β-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 a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
   Note: 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 min at room temperature.
   Note: 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 200–300 µl. It is normal to transfer some glass beads with the lysate.
6. Add 450 µl of Solution VN to the lysate. Vortex briefly on high to mix.
   Note: The sample may become opaque upon the addition of Solution VN. This is normal. Solution VN is a neutral salt solution that drives the association of proteins with the silica membrane surface.
7. Load up to 700 µl of the lysate/Solution VN mix into an MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. **Do not** discard the flow-through.
   Note: Proteins bind to the MB Spin Column silica filter in the presence of Solution VN.
8. Transfer the MB Spin Column to a clean 2 ml Collection Tube (provided).
9. Add 600 µl of Solution AB to the flow-through from step 7. Vortex briefly on high to mix.

Note: The flow-through can contain low molecular weight proteins that require alternative binding conditions. Adding Solution AB adjusts the binding conditions and promotes binding of low molecular weight proteins to the MB Spin Column filter membrane.

10. Load 650 µl of the mix from Step 9 into the same MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. Discard the flow-through and load the remaining sample volume into the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature and discard the flow-through.
    Note: The remaining proteins bind to the MB Spin Column filter membrane without displacing previously bound proteins.
11. Add 650 µl of RNase-Free Water to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. Discard the flow-through.
    Note: RNase-Free Water removes residual salts from the MB Spin Column filter membrane. Salt carryover will cause SDS precipitation in the final elution.
12. Centrifuge the empty MB Spin Column at 15,000 x *g* for 2 min at room temperature.
13. Being careful not to splash any liquid on the filter basket, place the MB Spin Column 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 Appendix A.
14. Add 100 µl of PE Solution to the center of the spin filter membrane. Incubate for a minimum of 1 min at room temperature.
    Note: Depending on the downstream application, alternate elution buffers, such as urea:thiourea or cleavable detergents, may be used. Using alternative elution buffers may result in reduced protein recovery. Refer to the Troubleshooting Guide for additional information.
    Note: 1% SDS is used to elute MB Spin Column 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 min at room temperature.

16. 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 1% SDS may be required. Refer to the Troubleshooting Guide for additional 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
Sample processing		
a)	Amount of sample to process	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 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 protein precipitation and may clog the MB Spin Column. Decreasing the amount of starting material may limit or prevent precipitation and thereby increase protein yields.
b)	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 Antifoam (provided) to Solution PL prior to bead beating (see directions on page 9 or 12). We recommend adding Antifoam to samples regardless of microbial species.
c)	Removing SDS	The elution buffer contains 1% SDS, which is required for maximum protein recovery from the MB Spin Column. For some downstream applications (e.g., 2D SDS-PAGE, in-solution proteolytic digestion), SDS will need to be removed. To keep proteins in solution, conduct a buffer exchange with 8 M urea, guanidine thiocyanate or other suitable chaotrope/detergent solutions using traditional dialysis tubing, a centrifugal filter device with low molecular weight cut-off (e.g., less than 5 kD) or a desalting column.

		Comments and suggestions
d)	Using alternative elution buffers	Proteins bound to the MB Spin Column can alternatively 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 (Waters), have also been shown to be effective alternatives to elution buffers but may reduce protein recovery. A ≥ 2% solution of *Rapi*Gest SF in Tris (pH 8) can serve as an alternative elution buffer.
Protein		
a)	Precipitating protein	The NoviPure Microbial Protein Kit two-step binding system is designed to sequentially immobilize a diverse range of proteins on MB Spin Column filter membranes. Deviating from the protocol by combining Solution VN and Solution AB or by 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.
b)	Degraded protein	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 Solution PL prior to bead beating. Follow preparation instructions found in the "Important points before starting" section (on page 9 or 12). Processing samples on ice may decrease protease activity but is not a recommended alternative due to the possibility of protein precipitation.
c)	Storing protein	For short term storage, proteins may be kept at 2–8°C. For long term storage, proteins may be stored at –15 to –30°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 protein samples at 55°C to re-solubilize the SDS prior to downstream applications.

Appendix A: On-Column Removal of Nucleic Acids

The NoviPure Microbial Protein Kit co-isolates nucleic acids. These nucleic acids are associated with proteins and will elute into the final sample. Nucleic acid carryover may negatively impact some downstream applications such as 2D electrophoresis.

The SDS present in Solution PE will inhibit nucleases, so we recommend the following on-column nuclease digestion protocol if you need to remove nucleic acids from samples. On-column treatment will allow enzymatic digestion of residual nucleic acids present in protein samples prior to elution from the MB Spin Column.

The DNase Max® Kit (cat. no. 15200-50) and RNase A enzyme (25 mg/ml) can be used to complete on-column nuclease digestion. Additional volumes of Solution AB and RNase-free water are required for this protocol and are provided with the NoviPure Microbial Protein Kit.

Protocol

1. Prepare 50 µl of 1X DNase Buffer for each sample by adding 5 µl of 10x DNase Buffer (from the DNase Max Kit) to 45 µl of RNase-Free Water.
2. Add 1 µl DNase I Enzyme (from the DNase Max Kit) and 1 µl RNase A to the 1x DNase Buffer from step 1.
3. Add 50 µl of the nuclease mix to the center of the MB Spin Column filter membrane after step 13 of the NoviPure Microbial Protein Kit protocol.
4. Incubate at room temperature for 15 min.
5. Add 600 µl of Solution AB to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. **Do not** discard the flow-through.
6. Load the entire flow-through into the same MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. Discard the flow-through.
7. Add 650 µl of RNase-Free Water to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. Discard the flow-through.

8. Centrifuge the MB Spin Column at 15,000 x *g* for 1 min at room temperature.
9. Being careful not to splash any liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
10. Proceed with step 14 of the NoviPure Microbial Protein Kit protocol.

Ordering Information

Product	Contents	Cat. no.
NoviPure Microbial Protein Kit (50)	For 50 preps: Protein extraction from microbial cell cultures	47044
Related products		
NoviPure Soil Protein Kit (20)	For 20 preps: Protein extraction from all soil types	30000-20
DNase Max Kit (50)	For 50 preps: Remove genomic DNA contamination in RNA preparations using a high activity DNase I enzyme and a highly specific DNase removal resin	15200-50
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
DNeasy UltraClean 96 Microbial Kit (384)	For 384 preps: High-throughput isolation of DNA from microbial cultures	10196-4
MagAttract® Microbial DNA Kit (384)	For 384 preps: Automated isolation of DNA from microbial and food cultures using automated processing and liquid handling systems	27200-4
DNeasy PowerFood® Microbial Kit (100)	For 100 preps: Isolation of inhibitor-free DNA from a variety of cultured foods	21000-100

Product	Contents	Cat. no.
DNeasy PowerLyzer® Microbial Kit (50)	For 50 preps: Isolation of high-quality DNA from microbial cultures using a bead-based homogenizer	12255-50
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.

Notes

Limited License Agreement for NoviPure Microbial Protein Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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