April 2018

NoviPure® Soil Protein Kit Handbook

For the extraction of protein from all soil types

Sample to Insight

QIAGEN

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Kit Contents

NoviPure Soil Protein Kit	**(20)**
Catalog no.	**30000-20**
Number of preps	**20**
NoviPure Bead Tubes	20
Solution SP1	315 ml
Solution SP2	32 ml
Collection Tubes (50 ml)	2 x 20
Low-Protein Binding Tubes (1.7 ml)	20
Quick Start Protocol	1

Storage

Solution SP1 and Solution SP2 should be stored at 2–8°C and used while they are cold. All other components of the NoviPure Soil 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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of NoviPure Soil Protein Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The NoviPure Soil Protein Kit is designed to extract extracellular and intracellular microbial protein from a wide range of soil types without co-extraction of interfering compounds, such as humic substances.

Principle and procedure

The NoviPure Soil Protein Kit's novel, two-buffer extraction protocol uses bead beating with a bead mix to efficiently lyse cells while solubilizing intracellular as well as extracellular protein. The end result is a cleaner protein pellet, free of most soil impurities when compared to protein extracted using traditional methods. The protein pellet can be resuspended in any buffer desired for further analysis or storage.

The NoviPure Soil Protein Kit protocol starts with the addition of 5 g of soil or sediment into a 50 ml NoviPure Bead Tube. The first solution is added along with user-supplied dithiothreitol (DTT). After a brief incubation on ice, the samples are homogenized using bead beating. The second solution is then added, and the bead tube is incubated on ice again, followed by another round of bead beating. The protein is precipitated, and the pellet is washed to remove residual salts and detergent. The pellet can then be resuspended in any number of buffers depending on the downstream application.

This protocol is designed for denatured protein extraction. If you are interested in native protein extraction, please contact QIAGEN Technical Support (**support.qiagen.com**).

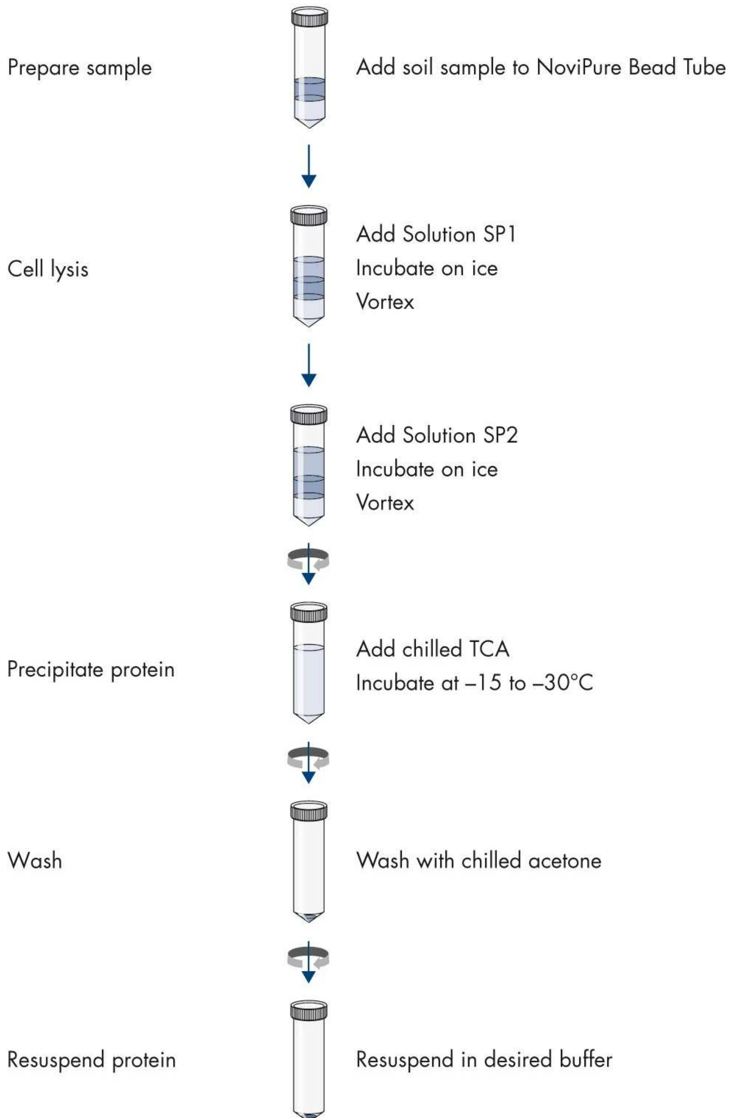

Figure 1. NoviPure Soil Protein Kit procedure.

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.

- Refrigerated centrifuge for 50 ml tubes (up to 4500 x *g*)
- Refrigerated microcentrifuge (up to 20,000 x *g*)
- Pipettors (100–1000 µl)
- Pipettes (5–25 ml)
- Vortex-Genie® 2
- Vortex Adapter for vortexing 50 ml tubes (cat. no. 13000-V1-50)
- Ice bucket
- Dithiothreitol (DTT), chilled to 2–8°C
- Trichloroacetic Acid (TCA), chilled to 2–8°C
- Acetone, HPLC-grade, chilled to –15 to –30°C

Protocol: Experienced User

Important points before starting

- Pre-chill Solutions SP1 and SP2 and a centrifuge to 2–8°C before starting. Shake to mix all solutions before use. All steps should be done with the samples kept on ice.
- HPLC-grade acetone should be stored at –15 to –30ºC. Keep cold for all washing steps.
- DTT can be added as a solid or as a solution. Solutions of DTT can become oxidized with exposure to air and do not remain stable over time. If using DTT solution, small amounts should be made, aliquoted and stored at –15 to –30ºC until ready to use.
  To prepare 10 ml stock solution of 1 M dithiothreitol (DTT), add 1.55 g of DTT to ddH_2O. Aliquot and freeze at –15 to –30ºC. Each sample will require 150 µl of 1 M DTT (or 0.023 g of solid DTT). Ensure that DTT solution is at 2–8°C during use.
- Prepare 100% (w/v) Trichloroacetic acid (TCA) by adding 227 ml of ultrapure or HPLC-grade water per 500 g of TCA. Each 1 ml of protein extract to be precipitated will require 0.25 ml of TCA. Store and use TCA solution while keeping it at 2–8°C.

Procedure

1. Add 5 g of soil to a NoviPure Bead Tube and place on ice.
2. Add 15 ml of Solution SP1 while keeping the NoviPure Bead Tube on ice.
3. Add 150 µl of 1 M DTT to the NoviPure Bead Tube (final concentration 10 mM).
4. Vortex or shake to mix completely and incubate on ice or at 2–8°C for 10 min.
5. Attach the NoviPure Bead Tube to a Vortex Adapter (cat. no.13000-V1-50) and vortex (in a cold room or refrigerator, if possible) at the highest speed for 10 min.
6. Quick-spin the NoviPure Bead Tube at 4500 x *g* in a refrigerated centrifuge (2–8°C) for 30 s to ensure residual soil/beads/buffer are removed from the top of the tube and cap.
7. Place the NoviPure Bead Tube back on ice and add 1.5 ml of cold Solution SP2.
8. Vortex or shake to mix completely and incubate on ice or at 2–8°C for 30 min.

9. Shake to mix and then attach the NoviPure Bead Tube to the Vortex Adapter and vortex (in a cold room or refrigerator, if possible) at the highest speed for 10 min.
10. Centrifuge at 4500 x *g* for 10 min in a refrigerated centrifuge (2–8°C).
11. Using a pipette, transfer supernatant to a clean, 50 ml Collection Tube (provided). Expect around 10 ml of supernatant from 5 g of soil.
    Optional: If extract still contains suspended soil particulates, repeat Steps 10 and 11.
12. Add 0.25 ml of 100% TCA for each 1 ml of supernatant to precipitate protein.
13. Vortex or shake briefly to mix and incubate at –15 to –30ºC for 1 h to overnight.
14. Thaw the 50 ml Collection Tube until the supernatant is liquid but still ice-cold.
15. Centrifuge the Collection Tube at 4500 x *g* in a refrigerated centrifuge (2–8°C) for 20 min. Pre-chill 1.7 ml Low-Protein Binding Tubes and a microcentrifuge to 2–8°C.
16. Remove as much liquid as possible without disturbing the pellet. If the pellet becomes dislodged, pipet off as much liquid as possible before proceeding. Discard the liquid.
17. Add 1 ml of ice-cold HPLC-grade acetone and completely resuspend the pellet by repeatedly pipetting and vortexing.
    Note: Keep samples on ice as much as possible and keep the acetone cold.
18. Transfer the acetone-suspended protein to a 1.7 ml Low-Protein Binding Tube (provided).
    Note: If a refrigerated centrifuge for 2 ml tubes is not available, the sample can be transferred to another 50 ml Collection Tube (user provided).
19. Centrifuge at 20,000 x *g* for 5 min in a refrigerated centrifuge (2–8°C). If a 50 ml Collection Tube was used in Step 18, centrifuge at 4500 x *g* for 10 min at 2–8°C.
20. Pour off the acetone while being careful to not dislodge the pellet. If the pellet becomes dislodged, remove acetone using a pipette tip.
21. Wash the pellet with 1 ml of ice-cold acetone and vortex for 10 s to resuspend.
22. Centrifuge at 20,000 x *g* for 5 min in a refrigerated centrifuge (2–8°C).
23. Repeat Steps 20–22. Then carefully pipet off the acetone, and dry the pellet in a hood or with N_2 gas until it is free of liquid but not crystallized.
    Note: Watch samples carefully. Pellets from soils high in organic material and darker pellets will take longer to dry (Drying time could vary from 5 to 60 min). When a pellet

is dry, it will pull away from the side of the tube. If a pellet becomes too dry, it will be difficult to resuspend and may also be lost from the tube.

Note: Keep dried pellets frozen (–15 to –30°C) until ready to proceed to next analysis.

24. Resuspend pellet in 25–200 µl of buffer of choice for downstream evaluation (e.g., Laemmli or Tris-HCl buffer for 1D gel visualization; ammonia bicarbonate, guanidine or urea buffer for 2D PAGE; trypsin digest prior to mass spectrometry).

    Note: Rigorous pipetting of the pellet is required to dissolve proteins. Please see Appendix A for specific buffer formulations.

    Note: The sample is now ready for 1D, 2D PAGE or 2D LC-MS/MS. Please see Appendix B if sample will be used for 1D LC-MS/MS.

Protocol: Detailed

Important points before starting

- Pre-chill Solutions SP1 and SP2 and a centrifuge to 2–8°C before starting. Shake to mix all solutions before use. All steps should be done with the samples kept on ice.
- HPLC-grade acetone should be stored at –15 to –30°C. Keep cold for all washing steps.
- DTT can be added as a solid or as a solution. Solutions of DTT can become oxidized with exposure to air and do not remain stable over time. If using DTT solution, small amounts should be made, aliquoted and stored at –15 to –30°C until ready to use. To prepare 10 ml stock solution of 1 M dithiothreitol (DTT), add 1.55 g of DTT to ddH_2O. Aliquot and freeze at –15 to –30°C. Each sample will require 150 µl of 1 M DTT (or 0.023 g of solid DTT). Ensure that DTT solution is at 2–8°C during use.
- Prepare 100% (w/v) Trichloroacetic acid (TCA) by adding 227 ml of ultrapure or HPLC-grade water per 500 g of TCA. Each 1 ml of protein extract to be precipitated will require 0.25 ml of TCA. Store and use TCA solution while keeping it at 2–8°C.

Procedure

1. Add 5 g of soil to a NoviPure Bead Tube and place on ice.
2. Add 15 ml of Solution SP1 while keeping the NoviPure Bead Tube on ice.
   Note: Solution SP1 is a gentle extraction buffer that contains a detergent that will not co-extract humic substances. Incubation on ice is necessary to maintain the desired pH, prevent protein degradation and improve protein recovery.
3. Add 150 µl of 1 M DTT to the NoviPure Bead Tube (final concentration 10 mM).
   Note: DTT is a disulfide-reducing agent. It both cleaves disulfide bonds and stabilizes thiol groups in proteins. It is important to use fresh DTT to inactivate proteases and protect proteins as they are being extracted.
4. Vortex or shake to mix completely and incubate on ice or at 2–8°C for 10 min.
   Note: Incubation in Solution SP1 before bead beating establishes a hypotonic environment that will improve mechanical cell lysis.

5. Attach the NoviPure Bead Tube to a Vortex Adapter (cat. no.13000-V1-50) and vortex (in a cold room or refrigerator, if possible) at the highest speed for 10 min.
   Note: Collision of the beads with microbial cells and extracellular protein bound to soil particles causes cell lysis and solubilization of proteins.
6. Quick-spin the NoviPure Bead Tube at 4500 x *g* in a refrigerated centrifuge (2–8°C) for 30 s to ensure residual soil/beads/buffer are removed from the top of the tube and cap.
   Note: The detergent contained in Solution SP1 results in foam formation. Quick-spinning the bead tube ensures residual soil, beads and buffer are removed from the top of the bead tube and cap before going on to the next step.
7. Place the NoviPure Bead Tube back on ice and add 1.5 ml of cold Solution SP2.
8. Vortex or shake to mix completely and incubate on ice or at 2–8°C for 30 min.
   Note: Incubation in Solution SP2 completes the lysis of microorganisms and improves intracellular and extracellular protein recovery.
9. Shake to mix and then attach the NoviPure Bead Tube to the Vortex Adapter and vortex (in a cold room or refrigerator, if possible) at the highest speed for 10 min.
   Note: A second bead beating step improves microbial cell lysis and extracellular protein recovery from the soil particles.
10. Centrifuge at 4500 x *g* for 10 min in a refrigerated centrifuge (2–8°C).
    Note: Extracted protein is separated from the soil particles and beads in the bead tube.
11. Using a pipette, transfer supernatant to a clean, 50 ml Collection Tube (provided). Expect around 10 ml of supernatant from 5 g of soil.
    Optional: If extract still contains suspended soil particulates, repeat Steps 10 and 11.
    Note: A second centrifugation step is occasionally required to separate fine soil particles from the extracted protein in solution. This extra centrifugation step will result in a cleaner protein pellet.
12. Add 0.25 ml of 100% TCA for each 1 ml of supernatant to precipitate protein.
    Note: Proteins are being precipitated out of solution. The supernatant will appear cloudy as a result of Solution SP1. Residual Solution SP1 will be removed during the acetone washes.

13. Vortex or shake briefly to mix and incubate at –15 to –30ºC for 1 h to overnight.
    Note: We recommend precipitating for a minimum of 1 h. Samples with high concentrations of protein will precipitate in an hour. For samples containing low protein concentrations, precipitate overnight.
14. Thaw the 50 ml Collection Tube until the supernatant is liquid but still ice-cold.
    Note: The sample must be thawed slowly and not heated. Samples may be thawed by holding the tubes or by placing them at 2–8°C for 30 min.
15. Centrifuge the Collection Tube at 4500 x *g* in a refrigerated centrifuge (2–8°C) for 20 min. Pre-chill 1.7 ml Low-Protein Binding Tubes and a microcentrifuge to 2–8°C.
    Note: Protein, solubilized organic material and residual Solution SP1 are pelleted at the bottom of the tube.
16. Remove as much liquid as possible without disturbing the pellet. If the pellet becomes dislodged, pipet off as much liquid as possible before proceeding. Discard the liquid.
    Note: Protein is now contained in the pellet at the bottom of the tube. The liquid is removed so that the pellet can be washed.
17. Add 1 ml of ice-cold HPLC-grade acetone and completely resuspend the pellet by repeatedly pipetting and vortexing.
    Note: Keep samples on ice as much as possible and keep the acetone cold.
    Note: The cold acetone solubilizes residual TCA and Solution SP1 but not the protein. Residual TCA and Solution SP1 need to be removed to prevent interference with downstream applications. The pellet will become smaller and more compact with each addition of cold acetone.
18. Transfer the acetone-suspended protein to a 1.7 ml Low-Protein Binding Tube (provided).
    Note: If a refrigerated centrifuge for 2 ml tubes is not available, the sample can be transferred to another 50 ml Collection Tube (user provided).
    Note: Transferring the resuspended protein pellet to a smaller tube results in a tighter pellet for easier handling during the subsequent wash steps. The Low-Protein Binding Tubes are safe to be used for mass spectrometry.
19. Centrifuge at 20,000 x *g* for 5 min in a refrigerated centrifuge (2–8°C). If a 50 ml Collection Tube was used in Step 18, centrifuge at 4500 x *g* for 10 min at 2–8°C.

Note: The protein is being pelleted again while residual TCA and Solution SP1 are being solubilized.

20. Pour off the acetone while being careful to not dislodge the pellet. If the pellet becomes dislodged, remove acetone using a pipette tip.
21. Wash the pellet with 1 ml of ice-cold acetone and vortex for 10 s to resuspend.
    Note: The protein pellet is being washed to remove residual TCA and Solution SP1.
22. Centrifuge at 20,000 x *g* for 5 min in a refrigerated centrifuge (2–8°C).
23. Repeat Steps 20–22. Then carefully pipet off the acetone, and dry the pellet in a hood or with N_2 gas until it is free of liquid but not crystallized.
    Note: The protein pellet is being washed a final time to remove residual TCA and Solution SP1. It should appear smaller and more compact than in previous steps.
    Note: Watch samples carefully. Pellets from soils high in organic material and darker pellets will take longer to dry (Drying time could vary from 5 to 60 min). When a pellet is dry, it will pull away from the side of the tube. If a pellet becomes too dry, it will be difficult to resuspend and may also be lost from the tube.
    Note: Keep dried pellets frozen (–15 to –30ºC) until ready to proceed to next analysis.
    Note: Residual acetone is being aerosolized and removed from the protein pellet. The protein pellet should be free of all traces of acetone but not dried too long or it will be difficult to resolubilize.
24. Resuspend pellet in 25–200 µl of buffer of choice for downstream evaluation (e.g., Laemmli or Tris-HCl buffer for 1D gel visualization; ammonia bicarbonate, guanidine or urea buffer for 2D PAGE; trypsin digest prior to mass spectrometry).
    Note: Rigorous pipetting of the pellet is required to dissolve proteins. Please see Appendix A for specific buffer formulations.
    Note: The sample is now ready for 1D, 2D PAGE or 2D LC-MS/MS. Please see Appendix B if sample will be used for 1D LC-MS/MS.

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 soil to process	The NoviPure Soil Protein Kit is designed to process 5 g of soil. Increasing the amount of soil will not improve relative yield for most soil types.
b)	Presence of keratin	Keratin is a family of fibrous structural proteins that make up hair, nails, hooves, wool, feathers and skin. Keratin is often abundant in soils, originating from mammals, birds, reptiles and some insects. Keratin is 55 to 65 kDa in size and can be visualized using SDS-PAGE. Keratin can mask the presence of rare proteins during mass spectrometry. Keratin contamination is most often introduced during protein purification steps but can also be introduced during protein extraction.
Protein		
a)	Storing protein	Protein stability (in solution or lyophilized) under different storage conditions is highly dependent on the specific proteins contained in the sample. For instance, some proteins may be stable when stored at 2–8°C while other proteins in the same sample may be degraded and instead require storage at –15 to –30°C or –65 to –90°C. The following recommendations are provided for mixed protein samples: For short term storage, proteins may be kept at 2–8°C for up to one month. We recommend that sodium azide (NaN_3) be added to a final concentration of 0.02–0.05% to prevent microbial contamination. For long term storage, proteins may be stored as a solution or lyophilized for several years. Samples should be aliquoted into single use quantities and stored frozen at –15 to –30°C, –65 to –90°C or in liquid nitrogen.

		Comments and suggestions
b)	Precipitating protein	Trichloroacetic acid (TCA) is a general precipitation reagent. We recommend that TCA be used for protein precipitation followed by acetone washing. Methanol-chloroform precipitation is not recommended and will result in a loss of protein.
c)	Quantifying protein	Depending on the soil type and the amount of solubilized organic material present in the extract, quantification of protein using a colorimetric type of assay (e.g., BCA, Bradford, Lowry's) may result in an inaccurate estimation. Protein quantification can be done after peptide digestion and sample desalting.

Appendix A: Buffer Formulations

- 50 mM Tris-HCl (pH 6.8–8.5)

  Add 6.1 g of Tris or Trizma to 900 ml of HPLC-grade or ultrapure water.
  Adjust pH to the desired level using concentrated HCl. Bring volume up to 1 liter.

- 50 mM Tris-$CaCl_2$ (pH 7.6)

  Add 6.1 g of Tris or Trizma and 1.11 g of $CaCl_2$ to 900 ml of HPLC-grade or ultrapure water.
  Adjust pH to 7.6 using concentrated HCl. Bring volume up to 1 liter.

- 25 mM ammonium bicarbonate

  Add 1.98 g of ammonium bicarbonate to HPLC-grade or ultrapure water to make 1liter.

- 2x Laemmli loading buffer

  Combine the following reagents:
  - 2.5 ml of 0.5 M Tris-HCl, pH 6.8
  - 2 ml of glycerol
  - 1 g of 10% (w/v) SDS
  - 0.01 g of 0.1% (w/v) Bromophenol Blue

  Bring the volume to 10 ml with HPLC-grade or ultrapure water.
  Add 50 µl of fresh β-Mercaptoethanol for every 1 ml of buffer needed.
  Dilute 1:1 with sample or water.

- 6 M guanidine HCl buffer

  Add 573.18 g of guanidine HCl and 1.5 g of dithiothreitol (DTT) to 420 ml of 50 mM Tris-$CaCl_2$, pH 7.6.
  Mix to dissolve and bring volume up to 1 liter with 50 mM Tris-$CaCl_2$, pH 7.6.

- 6 M urea buffer

  Add 4 g of urea (6 M) to 10 ml of 25 mM ammonium bicarbonate solution.

- 2D PAGE resuspension buffer

  Combine the following reagents:

  - 420.42 g of 7 M urea
  - 152.24 g of 2 M thiourea
  - 20 g of 2% (w/v) CHAPS
  - 20 ml of 2% Nonidet P-40
  - 20 mg of 0.002% (w/v) Bromophenol Blue
  - 5 ml of 0.5% ampholyte
  - 15.43 g of 100 mM 1,4-dithioerythritol (DTE)

  Bring the volume to 1 liter with HPLC-grade or ultrapure water.

Appendix B: Mass Spectrometry

Protein extracted using the NoviPure Soil Extraction Kit is suitable for mass spectrometry and has been evaluated using both 1D and 2D LC-MS/MS with excellent results. For 1D LC-MS/MS, a strong cation exchange (SCX) column is required for detergent removal after peptide digestion. Sample processing for 2D LC-MS/MS efficiently removes the detergent without additional cleanup.

The following protocols are for protein digestion, detergent removal and desalting. All three protocols are required for 1D LC-MS/MS. Only protein digestion and desalting are required for 2D LC-MS/MS.

Equipment required

- Ultrasonic bath
- Microcentrifuge (up to 20,000 x *g*)
- Pipettors (100–1000 µl)
- Vortex-Genie 2
- Strong cation exchange (SCX) column (Thermo Fisher Scientific cat. no. 90008)
- 10 ml syringe and needle
- Speed vac
- 2 ml tubes
- Screw cap tubes
- Shaking incubator for microcentrifuge tubes
- Sep-Pak® C18 column (Waters cat. no. WAT020515)

Reagents required

- 50 mM ammonium bicarbonate
- 3,3′,3″-Phosphanetriyltripropanoic acid (TECP) (Thermo Fisher Scientific cat. no. 77720)

- Iodoacetamide (IAA)
- pH strips
- Sequencing-grade trypsin (Promega® cat. no. V5111)
- 100% formic acid
- 0.1% formic acid
- 100% acetonitrile
- 0.1% formic acid/2% acetonitrile
- 0.1% formic acid/5% acetonitrile
- 0.1% formic acid/25% acetonitrile/500 mM potassium chloride
- 40% acetonitrile/0.06% formic acid

Trypsin digest

1. Add 400 µl of 50 mM ammonium bicarbonate to the pellet. Vortex to mix. The soil particles will not completely dissolve. Do not pipet up and down.
2. Place in an ultrasonic bath for 5 min.
3. Vortex to mix and return to the ultrasonic bath for an additional 5 min.
4. Vortex until all the soil particles are dissolved.
5. Add 8 µl of 0.5 M TCEP to a final concentration of 10 mM.
6. Vortex to mix. Incubate at 60°C for 30 min.
7. Add 16 µl of 0.5 M iodoacetamide (IAA) to a final concentration of 20 mM.
   Note: IAA must be made fresh and protected from the light. Wrap in foil and store at –15 to –30°C for up to 1 week.
8. Vortex to mix and incubate at 37°C with shaking for 30 min (800 rpm). Cover the shaker and sample with foil to protect the sample containing IAA from light.
9. Use a pH strip to ensure pH is at 8.0. If the starting material is a pellet and not a solution, then the pH will be within range. If the pH is out of range, then adjust with sodium bicarbonate ($NaHCO_3$).

10. Prepare trypsin according to the manufacturer's instructions. Resuspend the lyophilized pellet in 20 µl of buffer to make a 1 µg/µl stock. Aliquot and freeze at –15 to –30ºC.
11. Add trypsin at a 1:100 ratio of trypsin to protein. For example, for 400 µg of protein add 4 µl of trypsin stock solution (1 µg/µl).
12. Vortex to mix and incubate overnight at 37ºC with shaking (600 rpm). Cover with foil to protect the sample containing IAA from light.
13. Add 100% formic acid to a final concentration of 1% and vortex to mix.

Detergent removal using SCX columns

14. Add 400 µl of 0.1% formic acid/5% acetonitrile to an SCX column. Centrifuge at 2000 x *g* for 5 min. Discard the flow-through.
15. Centrifuge the sample (from step 13) at 14,000 x *g* for 1 min to pellet soil particles. Avoiding the pellet, add the sample to the column (from step 14). Centrifuge for 5 min at 2000 x *g*. Discard the flow-through.
16. Wash the column with 400 µl of 0.1% formic acid/5% acetonitrile. Centrifuge for 5 min at 2000 x *g*. Discard the flow-through and repeat the wash step a second time.
17. Transfer the column to a clean 2 ml tube.
18. Add 400 µl of a 50:50 mix of 0.1% formic acid/5% acetonitrile and 0.1% formic acid/25% acetonitrile/500mM potassium chloride. Centrifuge at 2000 x *g* for 5 min. Transfer eluate to a clean tube.
19. Add 400 µl of 0.1% formic acid/25% acetonitrile/500mM potassium chloride. Centrifuge at 2000 x *g* for 5 min. Transfer eluate to the tube used in step 18 to get a total of 800 µl of eluate.

    Note: Multiple elutions can be done using buffers with increasing salt concentrations.

Sample desalting

20. Using a needle and 10 ml syringe, load 10 ml of 100% acetonitrile into the syringe to wash it. Avoid forming air bubbles.
21. Remove the needle and dispense the 100% acetonitrile into a waste container.

22. Place the needle back on the syringe and load another 10 ml of 100% acetonitrile into the syringe. Avoid forming air bubbles.
23. Remove the needle and place the syringe into the top of a Sep-Pak C18 column and push the acetonitrile through with a fast drip count of 3–4 drops/second.
24. Place the needle back on the syringe and load 10 ml of 0.1% formic acid/ 2% acetonitrile. Avoid forming air bubbles.
25. Remove the needle and place the syringe into the top of the Sep-Pak C18 column and push through with a fast drip count.
26. Place the needle back on the syringe and load the sample into the syringe. Then load 1.6 ml of formic acid/2% acetonitrile into the same syringe. Avoid forming air bubbles.
27. Remove the needle and place syringe into the top of the Sep-Pak C18 column and push through in a slow drop-wise fashion. Do not push the sample through too quickly or the protein will not bind.
28. Place the needle back on the syringe and load 10 ml of 0.1% formic acid/ 2% acetonitrile. Avoid forming air bubbles.
29. Remove the needle and place the syringe into the top of the Sep-Pak C18 column. Wash the column by pushing through with a fast drip count.
30. Repeat steps 28 and 29. Then proceed to step 31.
31. Place the needle back on the syringe and load 1.6 ml of 40% acetonitrile/0.06% formic acid. Avoid forming air bubbles.
32. Remove the needle and place the syringe into the top of the Sep-Pak C18 column. Elute protein by pushing through with a slow drip count of 1–2 drops/second into a screw cap tube.
33. Using the needle poke 6–7 holes into the cap of the tube and place into a speed vac for 3–4 hours until all the liquid has evaporated.
34. Resuspend protein in 100 µl of 0.1% formic acid for loading onto mass spectrometry machines.

Ordering Information

Product	Contents	Cat. no.
NoviPure Soil Protein Kit (50)	For 20 preps: Protein extraction from all soil types	30000-20
Related products		
NoviPure Microbial Protein Kit (50)	For 50 preps: Protein extraction from microbial cell cultures	47044
RNeasy® PowerSoil® Total RNA Kit (25)	For 25 preps: Isolation of high-quality total RNA from all soil types	12866-25
DNeasy® PowerSoil Pro Kit (50)	For 50 preps: Isolation of microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For 250 preps: Isolation of microbial genomic DNA from all soil types	47016
DNeasy PowerSoil HTP 96 Kit (384)	For 4 x 96 preps: High-throughput isolation of DNA from soil samples in less than one day	12955-4
DNeasy PowerMax® Soil Kit (10)	For 10 preps: Isolation of microbial DNA from large quantities of soil; great for samples with low microbial load	12988-10
DNeasy PowerLyzer® PowerSoil Kit (50)	For 50 preps: Isolation of DNA from tough soil microbes; optimized for use with bead-based homogenizer	12855-50
DNeasy PowerLyzer PowerSoil Kit (100)	For 100 preps: Isolation of DNA from tough soil microbes; optimized for use with bead-based homogenizers	12855-100

Vortex Adapter	For vortexing 50 ml tubes using the Vortex-Genie 2	13000-V1-50

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