NoviPure™ Soil Protein Extraction Kit

Catalog No.	Quantity
30000-20	20 Preps

Instruction Manual

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Version: 06242013

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Introduction

The NoviPure™ Soil Protein Extraction 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. The novel, two buffer extraction protocol utilizes 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 traditional methods. The protein can be resuspended in any buffer desired for further analysis or storage. All reagents and plastics are certified protease- and protein-free.

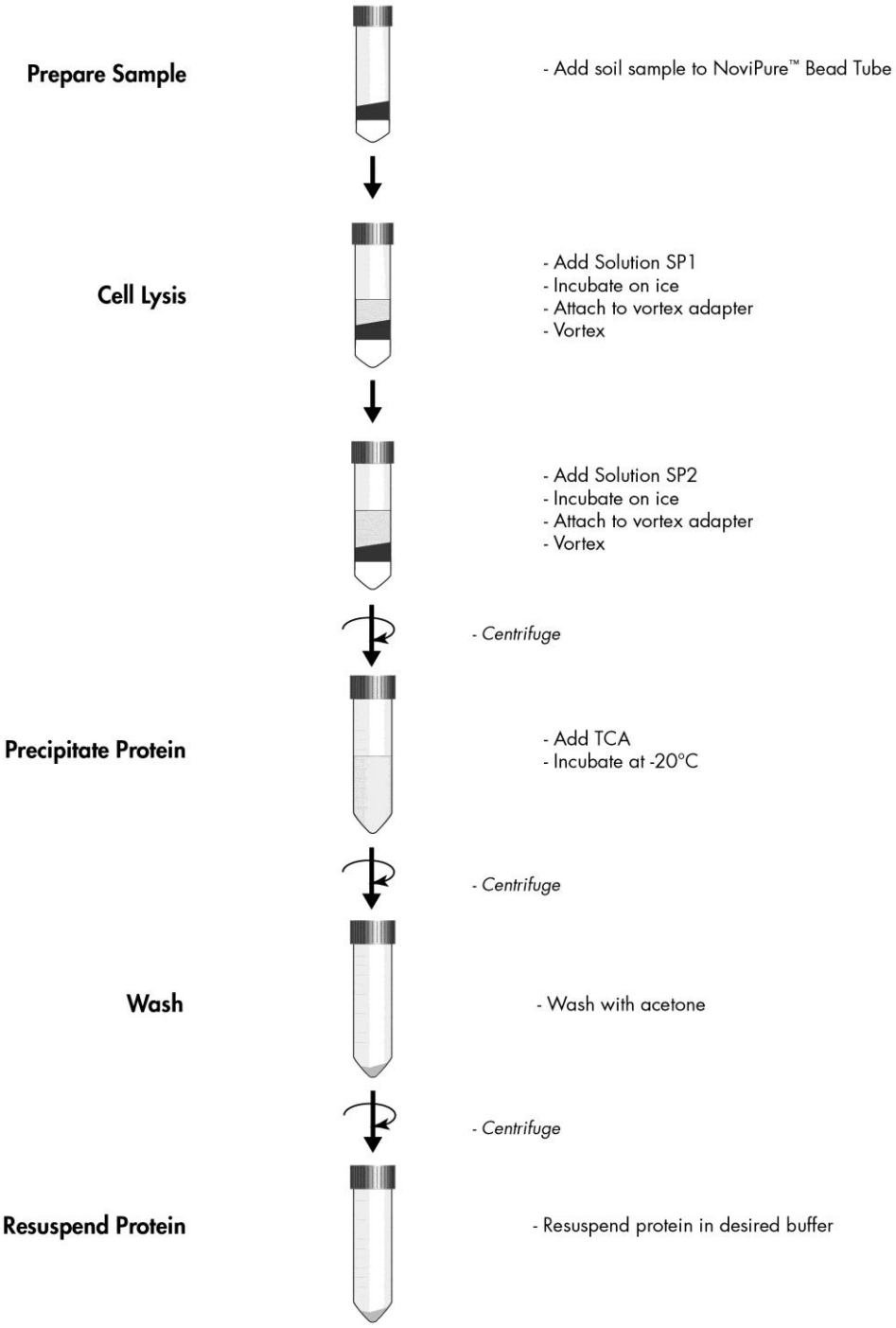
Protocol Overview

The NoviPure™ Soil Protein Extraction Kit starts with the addition of 5 g of soil or sediment into a 50 ml bead tube. The first solution is added along with user supplied dithiothreitol (DTT) and 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, contact technical support (technical@mobio.com).

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

Other Related Products	Catalog No.	Quantity
Vortex Genie® 2 Vortex	13111-V 13111-V-220	1 unit (120V) 1 unit (220V)
Vortex Adapter, holds 2 (50 ml) tubes	13000-V1-50	1 unit

NoviPure™ Soil Protein Extraction Kit



Equipment Required

Refrigerated Centrifuge for 50 ml tubes (≤4500 x *g*)
Refrigerated Microcentrifuge (20,000 x *g*)
Pipettors (100 µl - 1000 µl)
Pipets (5 ml - 25 ml)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
Vortex Adapter for 50 ml tubes (MO BIO Catalog# 13000-V1-50)
Ice bucket

Reagents Required But Not Included

Dithiothreitol (DTT)
Trichloroacetic Acid (TCA), chilled to 4ºC
Acetone, HPLC-Grade, cold at -20ºC

Kit Contents

	Kit Catalog# 30000-20
Component	**Amount**
NoviPure™ Bead Tubes	20 tubes
Solution SP1	315 ml
Solution SP2	32 ml
50 ml Falcon™ Collection Tubes	40
1.7 ml Low-Protein Binding Tubes	20

Kit Storage

Kit reagents should be stored at 4°C and used cold. All other 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 Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS 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.

Important Notes Before Starting

Pre-chill Solutions SP1, SP2 and the centrifuge to 4ºC before starting. All steps should be done with the samples kept on ice to protect the protein from degrading during extraction and precipitation.

Shake to mix all solutions before use.

HPLC-grade acetone should be stored at -20ºC and used cold for all washing steps.

Prepare 1M Dithiothreitol (DTT) solution (use cold at 4ºC)

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 -20°C until ready to use.

To prepare a 1M DTT solution, add 1.55 g of DTT to ddH_2O to make a 10 ml stock. Aliquot and freeze at -20°C. 150 µl of 1M DTT is required for each sample to be processed.

To use solid DTT, add 0.023 g to the bead tube.

Prepare 100% (w/v) Trichloroacetic acid (use cold at 4ºC)

Trichloroacetic Acid (TCA) is a general protein precipitation agent. TCA comes as a dry, crystalline chemical that needs to be reconstituted in water.

To prepare 100% (w/v) TCA, add 227 ml of ultrapure or HPLC water per 500 g of TCA. 0.25 ml of TCA is required for each 1 ml of protein extract to be precipitated.

TCA solution should be stored at 4°C.

Experienced User Protocol

Please wear gloves at all times

Pre-chill and maintain Solution SP1 and Solution SP2 at 4ºC. The HPLC-grade acetone should be stored at -20ºC. TCA should be used at 4ºC. All solutions should be used cold. Pre-chill all tubes before use.

1. Add 5 g of soil to the 50 ml NoviPure™Bead Tube (provided) and place the tube on ice.

2. Keeping the 50 ml NoviPure™Bead Tube on ice, add **15 ml of cold Solution SP1**.

3. Add **150 µl of 1M Dithiothreitol (DTT)** to the 50 ml NoviPure™ Bead Tube for a final concentration of 10mM. See page 6 for instructions on preparing DTT.
   Note: This protocol is for denatured protein extraction.

4. Vortex or shake to completely mix and incubate on ice or at 4°C for 10 minutes.

5. Attach the 50 ml NoviPure™ Bead Tube to the vortex adapter (MO BIO Catalog# 13000-V1-50) and vortex for 10 minutes at the highest speed.
   Note: Vortex in a cold room or refrigerator if possible.

6. Quick spin the 50 ml NoviPure™ Bead Tube at 4500 x *g,* in a refrigerated centrifuge set at 4°C for 30 seconds to ensure residual soil, beads, and buffer are removed from the top of the bead tube and cap.

7. Place the 50 ml NoviPure™ Bead Tube back on ice and add **1.5 ml of cold Solution SP2**.

8. Vortex or shake to completely mix and incubate on ice or at 4°C for 30 minutes.

9. Shake to mix the samples, then, attach the 50 ml NoviPure™ Bead Tube to the vortex adapter (MO BIO Catalog# 13000-V1-50) and vortex for 10 minutes at the highest speed.
   Note: Vortex in a cold room or refrigerator if possible.

10. Centrifuge the 50 ml NoviPure™ Bead Tube at 4500 x *g*, in a refrigerated centrifuge set at 4°C for 10 minutes.

11. Using a pipet, transfer supernatant to a clean, 50 ml Falcon™ Collection Tube (provided)
    Note: Expect around 10 ml of supernatant from 5 g of soil.

12. OPTIONAL: For extracts that still have suspended soil particulates, centrifuge again at 4500 x *g*, in refrigerated centrifuge set at 4°C for 10 minutes. Using a pipet, transfer supernatant to a clean, 50 ml Falcon™ Collection Tube (provided) avoiding any floating debris or touching the pellet.

13. Add 0.25 ml of 100% Trichloroacetic Acid (TCA) to each 1 ml of supernatant to precipitate the protein.
    Note: The extract will appear cloudy with the addition of TCA.

14. Vortex or shake briefly to mix and incubate at -20°C for 1 hour to overnight.

15. Thaw the 50 ml Falcon™ Collection Tube until the supernatant is liquid but still ice cold.

16. Centrifuge the 50 ml Falcon™ Collection Tube at 4500 x *g,* in a refrigerated centrifuge set at 4°C for 20 minutes to pellet the protein. Pre-chill the 1.7 ml Low-Protein Binding Tubes and a micro-centrifuge to 4°C. Make sure HPLC-grade acetone was stored at -20°C.

17. Remove as much of the liquid as possible without disturbing the pellet and discard the liquid.
    Note: Depending on the sample type, some pellets adhere to the side of the tube better than others. If the pellet becomes dislodged, pipette off as much of the liquid as possible and move to the next step. The acetone washes will help to remove residual buffer.

18. Add 1 ml of ice cold HPLC-grade acetone and completely resuspend the pellet by repeatedly pipetting and vortexing to disperse the pellet.
    Note: It is important at this step to keep the samples on ice as much as possible and keep the acetone cold to prevent resolubilization and potential loss of protein.

19. 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 then the sample can be transferred to another 50 ml Falcon™ Collection Tube (not provided). If another 50 ml Collection Tube is used then use the same centrifuge speeds (4500 x *g*) as in previous steps and increase the time to 10 minutes.

20. Centrifuge the 1.7 ml Low-Protein Binding Tube at 20,000 x *g*, in a refrigerated centrifuge set at 4°C for 5 minutes.

21. Pour off the acetone being careful to not dislodge pellet. If pellet becomes dislodged, remove acetone using a pipette tip.

22. Wash the pellet by adding another 1 ml of ice cold acetone and vortex 10 seconds to resuspend the pellet as much as possible.
    Note: The pellet does not need to be completely resuspended. Do not allow sample to remain off the ice for too long.

23. Centrifuge the 1.7 ml Low-Protein Binding Tube at 20,000 x *g*, in a refrigerated centrifuge set at 4°C for 5 minutes.

24. Pour off the acetone being careful to not dislodge pellet. If pellet becomes dislodged, remove acetone using a pipette tip.

25. Repeat the wash step by adding another 1 ml of ice cold acetone and vortex 10 seconds to resuspend the pellet as much as possible.
    Note: The pellet does not need to be completely resuspended. Do not allow sample to remain off the ice for too long.

26. Centrifuge the 1.7 ml Low-Protein Binding Tube at 20,000 x *g*, in a refrigerated centrifuge set at 4°C for 5 minutes.

27. Carefully pipet off the acetone and dry pellets in a hood or with N_2 gas until pellet is free of liquid but not crystallized.

Note: Watch the samples carefully at this point. The soil type and pellet color will influence how quickly the pellet dries. Soils that were high in organic material and yielded a darker pellet will take longer to dry. Drying time could be 5 minutes to an hour. When the pellet is dry, it will pull away from the sides of the tube. If the pellet becomes too dry it will be extremely difficult to resuspend. It can also be easily lost from the tube.

Note: Keep the dried protein pellet frozen at -20°C until you are ready to move to the next analysis.

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

    Protein pellets may be difficult to resuspend. Rigorous pipetting of the pellet is required to dissolve the proteins back into solution.

    Note: See the Appendix for specific buffer formulations.

    Your sample is now ready for 1D, 2D PAGE or 2D LC-MS/MS. For 1D LC-MS/MS, clean-up using a strong cation exchange (SCX) column is required. Please see the Hints and Troubleshooting guide for additional information.

    Thank you for choosing the NoviPure™ Soil Protein Extraction Kit.

Detailed User Protocol

Please wear gloves at all times

Pre-chill and maintain Solution SP1 and Solution SP2 at 4ºC. The HPLC-grade acetone should be stored at -20ºC. TCA should be used at 4ºC. All solutions should be used cold. Pre-chill all tubes before use.

1. Add 5 g of soil to the 50 ml NoviPure™ Bead Tube (provided) and place the tube on ice.

2. Keeping the 50 ml NoviPure™ Bead Tube on ice, add **15 ml of cold Solution SP1**.

   What is happening: 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 1M Dithiothreitol (DTT)** to the 50 ml NoviPure™ Bead Tube for a final concentration of 10mM. See page 6 for instructions on preparing DTT.
   Note: This protocol is for denatured protein extraction.

   What is happening: 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 the protein as it is being extracted.

4. Vortex or shake to completely mix and incubate on ice or at 4°C for 10 minutes.

   What is happening: Incubation in Solution SP1 before bead beating establishes a hypotonic environment that will improve mechanical cell lysis.

5. Attach the 50 ml NoviPure™ Bead Tube to the vortex adapter (MO BIO Catalog# 13000-V1-50) and vortex for 10 minutes at the highest speed.
   Note: Vortex in a cold room or refrigerator if possible.

   What is happening: 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 50 ml NoviPure™ Bead Tube at 4500 x *g,* in a refrigerated centrifuge set at 4°C for 30 seconds to ensure residual soil, beads, and buffer are removed from the top of the bead tube and cap.

   What is happening: 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 50 ml NoviPure™ Bead Tube back on ice and add **1.5 ml of cold Solution SP2**.

8. Vortex or shake to completely mix and incubate on ice or at 4°C for 30 minutes.

   What is happening: Incubation in Solution SP2 completes the lysis of microorganisms and improves intracellular and extracellular protein recovery.

9. Shake to mix the samples, then, attach the 50 ml NoviPure™ Bead Tube to the vortex adapter (MO BIO Catalog# 13000-V1-50) and vortex for 10 minutes at the highest speed.
   Note: Vortex in a cold room or refrigerator if possible.

   What is happening: A second bead beating step improves microbial cell lysis and extracellular protein recovery from the soil particles.

10. Centrifuge the 50 ml NoviPure™ Bead Tube at 4500 x *g*, in refrigerated centrifuge set at 4°C for 10 minutes.

    What is happening: Extracted protein is separated from the soil particles and beads in the bead tube.

11. Using a pipet, transfer the extract to a clean, 50 ml Falcon™ Collection Tube (provided)
    Note: Expect around 10 ml of supernatant from 5 g of soil.

12. OPTIONAL: For extracts that still have suspended soil particulates, centrifuge again at 4500 x *g*, in refrigerated centrifuge set at 4°C for 10 minutes. Using a pipet, transfer the extract to a clean, 50 ml Falcon™ Collection Tube (provided) avoiding any floating debris or touching the pellet.

    What is happening: 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.

13. Add 0.25 ml of 100% Trichloroacetic Acid (TCA) to each 1 ml of extract to precipitate the protein.
    Note: The extract will appear cloudy with the addition of TCA.

    What is happening: 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.

14. Vortex or shake briefly to mix and incubate at -20°C for 1 hour to overnight.

    What is happening: We recommend precipitating for a minimum of 1 hour. Samples containing a high concentration of protein will precipitate in an hour. For samples containing low protein concentrations, precipitation should be overnight.

15. Thaw the 50 ml Falcon™ Collection Tube until the extract is liquid but still ice cold.

    What is happening: The sample must be thawed slowly and not heated. Samples may be thawed by holding the tubes or by placing tubes at 4°C for 30 minutes.

16. Centrifuge the 50 ml Falcon™ Collection Tube at 4500 x *g*, in a refrigerated centrifuge set at 4°C for 20 minutes to pellet the protein. Pre-chill the 1.7 ml Low-Protein Binding Tubes and a micro-centrifuge to 4°C. Make sure HPLC-grade acetone was stored at -20°C.

    What is happening: Protein, solubilized organic material, and residual Solution SP1 are pelleted at the bottom of the tube.

17. Remove as much of the liquid as possible without disturbing the pellet and discard the liquid.
    Note: Depending on the sample type, some pellets adhere to the side of the tube better than others. If the pellet becomes dislodged, pipette off as much of the liquid as possible and move to the next step. The acetone washes will help to remove residual buffer.

What is happening: The protein is now contained in the pellet at the bottom of the tube and the liquid is removed so that the pellet can be washed.

18. Add 1 ml of ice cold HPLC-grade acetone and completely resuspend the pellet by repeatedly pipetting and vortexing to disperse the pellet.
    Note: It is important at this step to keep the samples on ice as much as possible and keep the acetone cold to prevent resolubilization and potential loss of protein.

    What is happening: 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.

19. Transfer the acetone suspended protein to a new 1.7 ml Low-Protein Binding Tube (provided).
    Note: If a refrigerated centrifuge for 2 ml tubes is not available then the sample can be transferred to another 50 ml Collection Tube (not provided). If another 50 ml Collection Tube is used then use the same centrifuge speeds (4500 x *g*) as in previous steps and increase the time to 10 minutes.

    What is happening: Transferring the resuspended protein pellet to a smaller tube results in a tighter forming pellet for easier handling during the subsequent wash steps. The Low-Protein Binding Tubes are safe for mass spectrophotometry.

20. Centrifuge the 1.7 ml Low-Protein Binding Tube at 20,000 x *g*, in a refrigerated centrifuge set at 4°C for 5 minutes.

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

21. Pour off the acetone being careful to not dislodge the pellet. If the pellet becomes dislodged, remove the acetone using a pipette tip.

22. Wash the pellet by adding another 1 ml of ice cold acetone and vortex 10 seconds to resuspend the pellet as much as possible.
    Note: The pellet does not need to be completely resuspended. Do not allow sample to remain off the ice for too long.

    What is happening: The protein pellet is being washed to remove residual TCA and Solution SP1.

23. Centrifuge the 1.7 ml Low-Protein Binding Tube at 20,000 x *g*, in a refrigerated centrifuge set at 4°C for 5 minutes.

24. Pour off the acetone being careful to not dislodge pellet. If pellet becomes dislodged, remove acetone using a pipette tip.

25. Repeat the wash step by adding another 1 ml of ice cold acetone and vortex 10 seconds to resuspend the pellet as much as possible.
    Note: The pellet does not need to be completely resuspended. Do not allow sample to remain off the ice for too long.

    What is happening: The protein pellet is being washed a final time to remove residual TCA and Solution SP1. The pellet should appear smaller and more compact than in previous steps.

26. Centrifuge the 1.7 ml Low-Protein Binding Tube at 20,000 x *g*, in a refrigerated centrifuge set at 4°C for 5 minutes.

27. Carefully pipet off the acetone and dry pellets at room temperature in a hood or with N_2 gas until pellet is free of liquid but not crystallized.

    Note: Watch the samples carefully at this point. The soil type and pellet color will influence how quickly the pellet dries. Soils that were high in organic material and yielded a darker pellet will take longer to dry. Drying time could be 5 minutes to an hour. When the pellet is dry, it will pull away from the sides of the tube. If the pellet becomes too dry, it will be extremely difficult to resuspend. It can also be easily lost from the tube.

    Note: Keep the dried protein pellet frozen at -20°C until you are ready to move to the analysis.

    What is happening: 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 the protein pellet will be difficult to resolubilize.

28. Resuspend pellet in 25 µl - 200 µl of the buffer of choice for downstream evaluation (*i.e.* Laemmli buffer or Tris–HCl buffer for 1D gel visualization or an ammonium bicarbonate, guanidine or urea buffer for 2D PAGE or trypsin digest prior to mass spectrophotometry).

    Protein pellets may be difficult to resuspend. Rigorous pipetting of the pellet is required to dissolve the proteins back into solution.

    Note: See the Appendix for specific buffer formulations.

    Your sample is now ready for 1D, 2D PAGE or 2D LC-MS/MS. For 1D LC-MS/MS, clean-up using a strong cation exchange (SCX) column is required. Please see the Hints and Troubleshooting guide for additional information.

    Thank you for choosing the NoviPure™ Soil Protein Extraction Kit.

Hints and Troubleshooting Guide

Amount of Soil to Process
This kit is designed to process 5 g of soil. Increasing the amount of soil will not improve relative yield for most soil types.

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 spectrophotometry. Keratin contamination is most often introduced during protein purification steps (Parker et al. 1998. Electrophoresis 19:1920-32) but can also be introduced during protein extraction.

All reagents and bead tubes have been tested for the presence of contaminating protein and are certified protein-free to ≤1 ng as well as protease-free.

Protein Precipitation
Trichloroacetic acid (TCA) is a general precipitation reagent. It is recommended 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.

Protein Quantitation
Depending on the soil type and the amount of solubilized organic material present in the extract, quantitation of protein using a colorimetric type of assay (*i.e.* BCA, Bradford, Lowry's) may result in an inaccurate estimation. Protein quantitation can be done after peptide digestion and sample desalting.

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 stored at 4ºC while other proteins in the same sample may be degraded and require storage at -20ºC or -80ºC. The following recommendations are provided for mixed protein samples.

For short term storage, protein can be kept at 4ºC for up to one month. It is recommended that sodium azide (NaN_3) be added to a final concentration of 0.02 – 0.05% to prevent microbial contamination.

For long term storage, protein can be stored as a solution or lyophilized for several years. Samples should be aliquoted into single use quantities and stored frozen at -20ºC, -80ºC or in liquid nitrogen.

Mass Spectrophotometry
Protein extracted using the NoviPure™ Soil Extraction Kit is suitable for mass spectrophotometry 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 clean-up.

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

Equipment Required

Ultrasonic bath (*i.e.* FisherScientific Catalog# FS30)
Pipettors (100µl - 1000 µl)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
Microcentrifuge (20,000 x *g*)
Strong Cation Exchange (SCX) column (ThermoFisher Pierce Catalog# 90008)
10 ml syringe and needle
Sep-Pak C18 column (Waters Catalog# WAT020515)
Speed Vac

Reagents Required But Not Included

50mM Ammonium Bicarbonate
3,3′,3″-Phosphanetriyltripropanoic Acid (TCEP)(Bond-breaker TCEP, Thermo Catalog# 77720)
Iodoacetamide (IAA)
Shaking incubator for microcentrifuge tubes (*i.e.* Thermomixer, Eppendorf)
pH strips
Sequencing Grade Trypsin (Promega Catalog# V5111)
100% Formic Acid
0.1% Formic Acid/5% Acetonitrile
0.1% Formic Acid/25% Acetonitrile/500mM Potassium Chloride
100% Acetonitrile
0.1% Formic Acid/2% Acetonitrile
40% Acetonitrile/0.06% Formic Acid
0.1% Formic Acid

Trypsin Digest

1. Add 400 µl of 50mM Ammonium Bicarbonate to the pellet. Vortex to mix. The soil particles will not completely dissolve. Do not pipette up and down.

2. Place tube in an ultrasonic bath for 5 minutes.

3. Vortex to mix and return to the ultrasonic bath for an additional 5 minutes.

4. Vortex until all the soil particles are dissolved.

5. Add 8 µl of 0.5M TCEP to a final concentration of 10mM.

6. Vortex to mix and incubate at 60ºC for 30 minutes.

7. Add 16 µl of 0.5M Iodoacetamide (IAA) to a final concentration of 20mM.
   Note: IAA must be made fresh and protected from the light. Wrap in foil and store at -20ºC for up to 1 week.

8. Vortex to mix and incubate at 37ºC with shaking for 30 minutes (800 RPM). Cover the shaker and sample with foil to protect the sample containing IAA from light.

9. Check pH to ensure it is at 8.0 using a pH strip. 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 (Sequencing Grade Trypsin, Promega, Cat#V5111). Resuspend the lyophilized pellet in 20 µl of buffer to make a 1 µg/µl stock. Aliquot and freeze at -20ºC.

11. Add Trypsin at a 1:100 ratio of trypsin to protein. For example, for 400 µg of protein add 4 µl of Trypsin (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 1% final concentration (4 µl) and vortex to mix.

Detergent Removal Using SCX columns

14. Add 400 µl of 0.1% formic acid/5% acetonitrile to the SCX column (ThermoFisher Pierce Catalog# 90008). Centrifuge at 2,000 x *g* for 5 minutes. Discard flow through.

15. Centrifuge the sample at 14,000 x *g* for 1 minute to pellet soil particles. Avoiding the pellet, add the sample to the column (~400 µl). Centrifuge at 2,000 x *g* for 5 minutes. Discard flow through.

16. Wash the column with 400 µl of 0.1% formic acid/5% acetonitrile. Centrifuge at 2,000 x *g* for 5 minutes. Discard flow through and repeat the wash step a second time.

17. Transfer the column to a clean 2 ml tube.

18. Elute twice with different salt concentrations as described below.

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

    Add 400 µl of 50:50 mix of (0.1% formic acid/5% acetonitrile) and (0.1% formic acid/25% acetonitrile/500mM potassium chloride). Centrifuge at 2,000 x *g* for 5 minutes. Transfer eluate to a clean tube.

    Add 400 µl of 0.1% formic acid/25% acetonitrile/500mM potassium chloride. Centrifuge at 2,000 x *g* for 5 minutes. Transfer eluate to the same clean tube to combine with the first elution. The final volume should be ~800 µl.

Sample Desalting

19. Using a needle and 10 ml syringe, load 10 ml of 100% acetonitrile into the syringe to wash it. Avoid the formation of air bubbles.

20. Remove needle and dispense the 100% acetonitrile into a waste container.

21. Place the needle back on the syringe and load another 10 ml of 100% acetonitrile into the syringe. Avoid the formation of air bubbles.

22. Remove the needle and place the syringe into the top of a Sep-Pak C18 column (Waters Catalog# WAT020515) and push the acetonitrile through with a fast drip count of 3 - 4 drops/second.

23. Place the needle back on the syringe and load 10 ml of 0.1% formic acid/2% acetonitrile. Avoid the formation of air bubbles.

24. Remove the needle and place the syringe into the top of the Sep-Pak C18 column and push through with a fast drip count.

25. Place the needle back on the syringe and load the sample (400 µl) into the syringe, followed by the loading 1.6 ml of 0.1% formic acid/2% acetonitrile into the same syringe. Avoid the formation of air bubbles.

26. 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.

27. Wash the column by placing the needle back on the syringe and load 10 ml of 0.1% formic acid/ 2% acetonitrile. Avoid the formation of air bubbles.

28. Remove the needle and place the syringe into the top of the Sep-Pak C18 column push through with a fast drip count.

29. Repeat Steps 27 and 28.

30. Elute protein by placing the needle back on the syringe and loading 1.6 ml of 40% acetonitrile/0.06% formic acid. Avoid the formation of air bubbles.

31. Remove the needle and place syringe into the top of the Sep-Pak C18 column and push through with a slow drip count of 1 – 2 drops/second into a screw cap tube.

32. 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 is evaporated.

33. Resuspend the protein in 100 µl of 0.1% formic acid for loading onto the mass spec machine.

Appendix: Buffer Formulations

50mM Tris-HCl (pH 6.8 to 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 1L.

50mM 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 1L.

25mM Ammonium Bicarbonate

Add 1.98 g of Ammonium Bicarbonate to HPLC-grade or ultrapure water to make 1L.

2X Laemmli Loading Buffer

Combine the following reagents:

0.5M Tris-HCl, pH 6.8	2.5 ml
Glycerol	2 ml
10% (w/v) SDS	1 g
0.1% (w/v) Bromophenol Blue	0.01 g

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

6M Guanidine HCl Buffer

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

6M Urea Buffer

Add 4 g of Urea (6M) to 10 ml of 25mM Ammonium Bicarbonate solution.

2D PAGE Resuspension Buffer

Combine the following reagents:

7M Urea	420.42 g
2M Thiourea	152.24 g
2% CHAPS (w/v)	20 g
2% Nonidet P-40	20 ml
0.002% Bromophenol Blue (w/v)	20 mg
0.5% Ampholyte	5 ml
100mM 1, 4-Dithioerythritol (DTE)	15.43 g

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

Contact Information

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Description	Catalog No.	Quantity
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
RNA PowerSoil® Total RNA Isolation Kit	12866-25	25 preps
RNA PowerSoil® DNA Elution Accessory Kit	12867-25	25 preps
PowerMax® Soil DNA Isolation Kit	12988-10	10 preps
PowerLyzer™ PowerSoil® DNA Isolation Kit	12855-50 12855-100	50 preps 100 preps
PowerSoil®-htp 96 Well Soil DNA Isolation Kit	12955-4 12955-12	4 x 96 preps 12 x 96 preps
PowerMag® Soil DNA Isolation Kit (Optimized for KingFisher®)	27000-4-KF	4 x 96 preps
PowerMag® Soil DNA Isolation Kit (Optimized for epMotion®)	27100-4-EP	4 x 96 preps