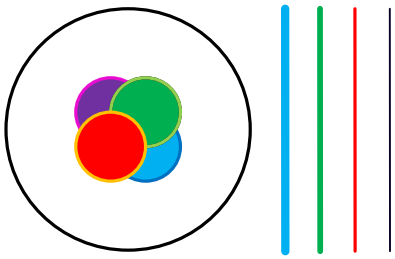
Biopolymer Isolation Technologies

THE POWER OF CHEMISTRY IN BIOLOGY

Soil DNA Isolation Kit

Direct precipitation of nucleic acids
Bead tubes with 3 different bead types in 5 different sizes
Use Lysis Buffer to store and transport samples; up to 7days
Directly precipitate nucleic acids from lysate
Patent pending pellet wash

Lyse'N'Pellet Soil DNA Isolation Kit



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Mini Prep

Trial Size	**10 Preps**	**SLDMN-01-10**
Regular Size	**50 Preps**	**SLDMN-01-50**
Regular Size	**100 Preps**	**SLDMN-01-100**

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Lyse'N'Pellet Approach

The homogenization efficiency is significantly improved by using high density beads. The composition includes our specialized beads with a density of 6.2 g/cc, steel beads with a density of 7.9 g/cc and zirconia with a density of 5.5 g/cc.

Bead Tubes contain five different sizes of beads in defined proportions. This is to improve lysing efficiency of cells. Environmental samples contain living cells that vary in sizes over a wide range and having beads in different sizes will improve efficiency of cell lysis. This range in bead sizes will also help to break soil particles and release trapped microbial cells with in them.

The utility of Lysis Buffer has been significantly improved to include sample storage, resuspension and lysis of microbial cells. Lysis Buffer minimizes solubilization of phenolic compounds present in soil samples and at the same time solubilizes proteins. This approach simplifies the protocol by removing the need for multiple precipitation steps and reduces hands-on time.

Alcohol precipitation preferentially precipitates both nucleic acid and humic acid, while in phenol:chloroform purification, humic acids separate out into the aqueous phase along with nucleic acids. Polyethyelene glycol (PEG) has been used instead of alcohol to precipitate nucleic acids. This protocol is time consuming and residual PEG inhibits PCR. Other specialized purifications have their own disadvantages, the main one being loss of nucleic acids during sample purification. Gel filtrations are expensive and result in loss of nucleic acids based on fragment sizes. Chemical flocculation using multivalent cations are effective, however, their binding is not specific and results in loss of nucleic acids based on its concentration, in a non-specific manner.

Our protocol is simple and straight forward. We precipitate total nucleic acids by a five-minute precipitation step and purified by washing the pellets with our patent pending Wash Buffer I. The pellets are washed again with Wash Buffer II, to remove residual Wash Buffer I. Pellets are resuspended and nucleic acids are selectively released in Elution Buffer. There are no lengthy incubations and extended centrifugation for nucleic acid precipitations. Soil particles, nucleic acids, proteins and humic acids are all charged biopolymers. They interact with each other, depending on the surface area, ionic strength of the solution and pH. In a natural sample such as soils, quantifying these interactions is extremely difficult.

Kit Contents

Components	Trial Kit 10 Preps	Regular 50 Preps	Regular 100 Preps
Bead Tubes	10	50	100
1.5 ml Collection Tubes	30	150	300
2 ml Collection Tubes	10	50	100
Lysis Buffer	15 ml	70 ml	140 ml
Bind Buffer	12 ml	60 ml	120 ml
Wash Buffer I	15 ml	80 ml	170 ml
Wash Buffer II	18 ml	85 ml	170 ml
Elution Buffer	5 ml	15 ml	30 ml

Reagent Information	Lysis Buffer	Bind Buffer	Wash Buffer I	Wash Buffer II	Elution Buffer
Room Temperature Storage	Yes	Yes	Yes	Yes	Yes
Flammable Reagent	No	Yes	No	Yes	No
Contact with Bleach	Ok	Ok	Ok	Ok	Ok
Regular Disposal	Yes	Yes	Yes	Yes	Yes
Sterilized	No	No	No	Yes	No

Quick Protocol

1. Add up to 250 mg of soil samples in to Bead Tubes and add 1.1 ml of Lysis Buffer. Vortex for about 10 seconds to resuspend the soil mass.

2. Homogenize soil samples either by vortexing for 5 minutes or using a bead beater as per manufacturers' instructions.

3. Centrifuge Bead Tubes at 7,000 x *g* for 1 minute and transfer the supernatant to a 1.5 ml collection tube.

4. Centrifuge at 10,000 x *g* for 5 minutes and transfer the supernatant to a 2 ml collection tube.

5. Add 1.1 ml of Bind Buffer and invert at least 10 times.

6. Centrifuge at 10,000 x *g* for 10 minutes and discard the supernatant, making sure to retain the pellet.

7. Add 500 µl of Wash Buffer I and resuspend the pellet by pipetting up and down. Centrifuge at 10,000 x *g* for 3 minutes and discard the supernatant.

9. Wash the pellets with Wash Buffer II. Add 500 µl of Wash Buffer II and invert the tubes gently, at least 5 times. Centrifuge at 10,000 x *g* for 5 minute. Discard the supernatant and centrifuge one more time at 10,000 x *g* for 1 minute to collect the residual Wash Buffer II. Remove the collected Wash Buffer II carefully and air dry the pellets for 10 to 15 minutes, or using a speedvac.

10. Resuspend the pellets in 125 µl of Elution Buffer and centrifuge one more time at 10,000 x *g* for 1 minute. Depending on your sample type, you may see a pellet, without disturbing the pellet remove the supernatant to a 1.5 ml collection tube. If need be, centrifuge one more time and transfer the superntant to yet another 1.5 ml tube.

1. **Add up to 250 mg of soil samples to Bead Tubes followed by 1.1 ml of Lysis Buffer. Vortex the Bead Tubes for at least 10 seconds to resuspend the soil mass.**

The weight of soil mass is primarily determined by its chemical composition and water holding capacity. For compost and commercial potting soil, please use up to 10 mg. If you have sediment slurry, it will be easier to centrifuge the slurry at high speed for a few minutes, remove water and use the pellet for DNA extraction. **You can collect, store and transport your soil samples in Lysis Buffer for up to 7 days before proceeding with extraction.**

2. **Homogenize soil samples either by vortexing for 5 minutes or using a bead beater as per manufacturers' instructions.**

Manual Vortexing	This is the cheapest mode of homogenizing your samples. You can hand-vortex for up to 5 minutes by holding the tube up right on a vortexer or tape the tubes to a flat bed vortexing platform (usually 3" diameter) that comes with vortexers. You can use autoclave tapes or rubber bands to hold the tubes horizontally on flat bed platforms.
Vortex Adaptors	You can homogenize your samples using vortex adaptors for up to 5 minutes.
Homogenizers	The Bead Tubes are compatible with a variety of homogenizers currently available in the market. Please refer to manufacturers' guide for time and speed.

3. **Centrifuge the Bead Tubes at 7,000 x *g* for 1 minute and decant the supernatant into a 1.5 ml collection tube. Centrifuge one more time at 10,000 x *g* for 5 minutes. Transfer the supernatant to a 2 ml collection tube without disturbing the pellet.**

Decant supernatant without any floating debris. This step will pellet out fine soil particles and separate out non-soil debris. Removing fine soil particles is essential because of their ability to easily bind with nucleic acids. Slower centrifugation of bead tubes prevents sedimenting of soil bound nucleic acid along with soil particles.

4. **Add 1100 µl of Bind Buffer and invert at least 10 times to mix. Centrifuge at 10,000 x *g* for 10 minutes and decant the supernatant carefully without disturbing the pellet.**

This quick centrifugation precipitates nucleic acids instantaneously.

5. **Resuspend the pellets in 500 µl of Wash Buffer I. Vortex the tubes vigorously to resuspend the pellet. Centrifuge at 10,000 x *g* for 3 minutes and discard the supernatant.**

The pellets may not completely resuspend in the Wash Buffer I. Resuspending in Wash Buffer I will neutralize the soluble phenolic compounds and prevents it from eluting along with nucleic acids.

6. **Add 1 ml of Wash Buffer II and gently invert the tubes at least 5 times and centrifuge at 10,000 x *g* for 5 minutes and discard the supernatant carefully without disturbing the pellet. Centrifuge one more time at 10,000 x *g* for 1 minute to collect the residual Wash Buffer II at the bottom of the tube. Gently pipette out the collected Wash Buffer II and air dry pellets for about 10 to 15 minutes at room temperature.**

Centrifugation will pellet both organic debris and nucleic acids, while washing with Wash Buffer II will remove residues of Wash Buffer I and any salt that precipitated out along with nucleic acids.

7. **Resuspend pellets in 125 µl of Elution Buffer. Vortex vigorously to break open the pellet, if necessary. Centrifuge at 10,000 x *g* for 1 minute and carefully transfer the supernatant to a 1.5 ml collection tube.**

Depending on your soil type, you may see a pellet. If you accidently touched the pellet or if the pellet is loose, re-centrifuge one more time to avoid removing the pellet.

Soil Sample

The exceptional microbial diversity and intrinsic variations found in structural organization of soil matrix will influence the amount of soil samples used in DNA extractions. DNA yields were positively correlated with sample size for sandy and silt soils, whereas no correlation was observed in clay soils. The recommended amount of soil in our protocol is between 50 - 250 mg and you can use the following information as an aid to guide your choice of the amount of starting material:

Sand and Silt	250 mg
Clay	100 mg
Compost	50 mg
Sediments	200 mg*
Commercial Potting Mixture	150 mg
Bog Soil	100 mg
Mulch	200 mg

* If you are using a watery sediment, it may be necessary to centrifuge your samples at high speed (> 10,000 x *g* for at least 2 minutes) and discard the supernatant water samples, and sample from the pellet.

Soil Extraction & Loss of Lysis Buffer

During extraction with lysis buffer, based on the water content of your samples, there will be a loss of up to 200 µl of lysis buffer. In some cases such as dry clay, potting mixtures, mulch, etc., this loss will be pronounced. You can continue with your prep and will not affect your extraction efficiency. However if the loss is pronounced, then you can reduce the amount of starting material to between 100 mg to 200 mg or use our Midi prep which can handle up to 2 g of soil sample.

Low Yield

Try a range of soil amounts from 50 mg to 250 mg. Poor yield is usually due to low biomass load or due to excessive organic content of soil matrix. Increasing the sampling amount will improve DNA yield in soils low in biomass and decreasing the sampling amount will improve DNA yield in soils high in organic matter.

Low yield and changes in the quality of DNA will result from changing the proportions of Lysis Buffer and Bind Buffers. The homogenization time and centrifugation speed can be changed ± 20% depending on the nature of your sample and the type of instrument being used by customers.

Poor Quality DNA

Soil DNA is co-extracted with impurities that inhibit enzyme activities making DNA unsuitable for any downstream application.

One easy option is to re-bind the eluted DNA back to a spin column in the presence of Bind Buffer.
For one volume of eluted DNA (eg. 100 µl) , add two volumes of (200 µl) of Lysis Buffer and at least three Volumes (300 µl) of Bind Buffer and load onto spin columns, wash with 500 µl of 70% ethnaol and elute DNA in desired volume of sterile water.

If you do not have a spin column, you can purify DNA by precipitating it out in the presence of our Bind Buffer:
To one volume of eluted DNA, add one volume of Lysis Buffer and at least six volumes of Bind Buffer.

Incubate at room temperature for at least 5 minutes and centrifuge at 10,000 x *g* for 10 minutes.

Decant the supernatant and wash the pellet in 1 ml of 70% ethanol (add 1 ml of 70% ethanol and invert the tubes at least five time and centrifuge at 10,000 x *g* for at least 5 minutes and decant the supernatant).

Air dry the pellet and resuspend in a desired volume of Elution Buffer.

Centrifuge again at 10,000 x *g* for 1 minute and remove the supernatant to another elution tube. If you see a brown pellet at the bottom of the tube, do not disturb the pellet.

You can also use the above procedure to concentrate eluted DNA.