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Fisher BioReagents®

SurePrep™ Soil DNA Isolation Kit

Product Cat. # BP2815-50

Instruction Manual

I. Introduction

A. Product Description

The SurePrep™ Soil DNA Isolation Kit provides a convenient and rapid method for the detection of microorganisms from soil samples. All types of soil samples can be processed with this kit, including common soil samples and difficult soil samples with high humic acid content such as compost and manure. The kit removes all traces of humic acid using the provided beads and a combination of chemical and physical homogenization and lysis. A simple and rapid spin column procedure is then used to further purify the DNA. Total genomic DNA can be isolated and purified from all the various microorganisms found in soil, such as bacteria, fungi and algae. The purified DNA is of the highest quality and is fully compatible with downstream PCR applications, since all humic acid substances and PCR inhibitors are removed during the isolation.

B. Overview of Procedure

Purification is based on spin column chromatography using Fisher's proprietary resin as the separation matrix. The process involves first measuring the soil sample into a provided 2 mL Screw Cap Tube, and adding an appropriate amount of the provided Beads. Next, Lysis Solution is added to the tube and vortexed briefly to mix. Lysis Additive is then added and the tube is vortexed for 5 minutes in order to efficiently and rapidly homogenize the sample, extract the DNA and remove all humic acids. The sample is then centrifuged, and the supernatant is transferred to a DNase-free microcentrifuge tube. Binding Solution is added, and the lysate is incubated for 5 minutes on ice. The lysate is then spun for 5 minutes to pellet any cell debris, the supernatant is collected, an equal volume of ethanol is added to the lysate and the solution is loaded onto a spin-column. Fisher's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while the proteins are removed in the flowthrough or retained on top of the resin. The bound DNA is then washed using the provided Wash Solutions, and the purified DNA is eluted using the Elution Buffer. The purified total DNA is free of all inhibitors, including humic acid, and can be used in sensitive downstream applications such as PCR.

C. Kit Specifications

Kit Specifications	
Maximum Soil Input: Clay, loam and sand Feces and compost	 250 mg 100-150 mg
Type of Soil Processed	All types, including common soil, compost and manure
Column Binding Capacity	50 μg
Maximum Column Loading Volume	600 μL
Time to Complete 10 Purifications	30 minutes

D. Advantages

- Rapid and convenient method to detect microorganisms in soil samples
- Process all types of soil, including common soil, compost and manure
- Remove all humic acid from DNA samples
- Fast and easy processing using a rapid spin-column format
- Isolate high quality total DNA from a variety of microorganisms including bacteria, fungi and algae

E. Kit Components

Component	Catalog # BP2815-50 (50 preps)
Lysis Solution	45 mL
Lysis Additive	6 mL
Binding Solution	6 mL
Wash Solution I	30 mL
Wash Solution II	18 mL
Elution Buffer	6 mL
Beads	30 g
Screw Cap Tubes (2 mL)	50
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

F. Storage Conditions and Product Stability

All kit solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 2 years in their unopened containers.

G. Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDS). The MSDS can be requested through our Customer Service Department.

H. Customer-Supplied Reagents and Equipment

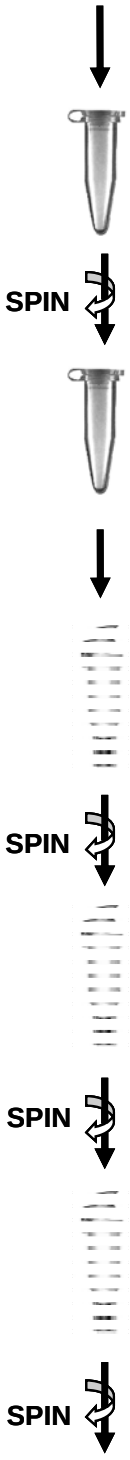
You must have the following in order to use the SurePrep™ Soil DNA Isolation Kit:

- Benchtop microcenrifuge
- DNase-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment
- 95-100% ethanol
- 70% ethanol

Flowchart

Procedure for Purifying Total DNA Using the SurePrep Soil DNA Isolation Kit

Add beads, soil sample, Lysis Solution and Lysis Additive to screw cap tube



Purified Total DNA

II. Set-Up and Preparation of Sample Lysate

A. Equipment Preparation

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\ (r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

If you do not own a variable speed microcentrifuge consider purchasing Thermo Scientific's Sorvall Legend benchtop model that offers power, safety and convenience.

- Choice of 17,000 or 21,000 x g (for RNA purification using SurePrep kits, the microcentrifuge with 17,000 x g is sufficient)
- Holds 36 x 0.5 mL microtubes, 24 x 2 mL tubes or 8 x 8 PCR
- Unique ClickSeal™ bio-containment rotor lid for safe processing of infectious specimens
- Fast acceleration and deceleration speeds up your protocols
- Broad range of rotors supports virtually any application
- Intuitive controls and vivid display
- Highly resistant materials allow vigorous cleaning and autoclaving

Sorvall Legend Micro Centrifuges

Technical Specifications

	Sorvall Legend Micro 17 & 17R	**Sorvall Legend Micro 21 & 21R**
Max g-force:	17,000	21,100
Max RPM:	13,300	14,800
Noise level:	<55 dBA	<56 dBA
Time set range:	1 min - 99 min; 1 min increments	1 min - 99 min; 1 min increments
Temp set range:	Set from -9 °C to +40 °C; per 1 °C increment	Set from -9 °C to +40 °C per 1 °C increment

Ordering Information

	Cat. No.	Cat. No.
Sorvall Legend Micro 17/17R	230V 50/60Hz	120V 60 Hz
Sorvall Legend Micro 17, includes 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002430	75002431
Sorvall Legend Micro 17R,includes 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002440	75002441
Sorvall Legend Micro 21/21R	230V 50/60Hz	120V 60 Hz
Sorvall Legend Micro 21, incl. 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002435	75002436
Sorvall LegendMicro 21R, incl. 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002445	75002446

For detailed product specifications, information on additional rotors, lids and adapters visit **www.thermo.com**

B. Preparation of Lysate from Soil Samples

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at **14,000 x g (~12,000 RPM)** except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of **Wash Solution II** by adding 42 mL of 95 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution II**. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Recommended soil input varies depending on the soil type. For clay, loam and sand the recommended soil input is 250 mg. For fecal samples and compost, it is recommended that 100-150 mg of sample be used.

Lysate Preparation

a. Weigh up to 250 mg of soil sample (please see Notes Prior to Use) into a 2 mL **Screw Cap Tube** provided with the kit.

   Note: In case of a wet soil sample, transfer the sample to a clean 1.7 mL microcentrifuge tube and centrifuge for 30 seconds at **14,000 × g (~12,000 RPM)**. Remove the water carefully using a pipette, and weigh the remaining soil.

b. Add a 2:1 ratio of the provided **Beads** into the screw cap tube with the soil (500 mg of glass beads is added to 250 mg of soil).
c. Add 700 µL of **Lysis Solution** to the tube. Vortex briefly to mix soil and Lysis Solution.
d. Add 100 µL of **Lysis Additive** and vortex briefly.
e. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g. Scientific Industries' Disruptor Genie™). Vortex for 5 minutes at maximum speed.
f. Centrifuge the tube for 1 minute at **14,000 × g (~12,000 RPM)**.
g. Transfer up to 450 µL of supernatant to a DNase-free microcentrifuge tube (not provided).
h. Add 100 µL of **Binding Solution**, mix by inverting the tube a few times, and incubate for 5 minutes on ice.
i. Spin the lysate for 1 minute to pellet any protein and soil particles.
j. Using a pipette, transfer up to 450 µL of supernatant into a DNase-free microcentrifuge tube (not provided).

Note: Avoid any contact with the pellet when collecting the supernatant. Also, depending on the soil type, some residue may be present on top of the supernatant. It is important to avoid collection of this residue while collecting the supernatant.

k. Add an equal volume of 70% ethanol (provided by the user) to the lysate collected above (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. Proceed to step **III. A Binding DNA to Column.**

III. Purifying DNA from Soil Sample Lysates

A. Binding DNA to Column

a. Assemble a **spin column** with one of the provided **collection tubes**.
b. Apply up to 600 μL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **14,000 × g (~12,000 RPM**). Discard the flowthrough and reassemble the spin column with the collection tube.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

c. Depending on your lysate volume, repeat step **b** if necessary.

B. Column Wash

a. Apply 500 μL of **Wash Solution I** to the column and centrifuge for 1 minute.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Apply 500 μL of **Wash Solution II** to the column and centrifuge for 1 minute.
d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Repeat **c** and **d**.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

C. DNA Elution

a. Place the column into a fresh 1.7 mL **Elution Tube** provided with the kit.
b. Add 50 μL of **Elution Buffer** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~1,500 RPM)**, followed by a one minute spin at **14,000 x *g* (~12,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~12,000 RPM) for one additional minute.
d. **(Optional)** An additional elution may be performed if desired by repeating steps **b** and **c** using 50 μL of **Elution Buffer**. The total yield can be improved by an additional 20-30% when this second elution is performed.

D. Assessing DNA Yield by UV Absorbance

Spectrophotometric measurement of the amount of UV irradiation absorbed by DNA is simple and accurate when the DNA sample is pure.

a. Determine DNA concentration by making an appropriate dilution of the purified DNA solution in TE (10 mM Tris and 1 mM EDTA, pH 7.4). Measure absorbance of the diluted sample in a 1 mL cuvette using a traditional UV-VIS spectrophotometer at 260 and 280 nm. The spectrophotometer should first be zeroed with the TE used to dilute the sample.
b. An A_{260} of 1.0 is equivalent to 50 μg double-stranded DNA per mL. Calculate the DNA concentration in μg/mL as follows:

A_{260} x dilution factor x 50 = μg DNA/mL

c. The ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the DNA purity with respect to contaminants that absorb in the UV range such as protein. Ratios of 1.8 to 2.0 indicate highly purified preparations of DNA. Contaminants such as protein that absorb at 280 nm will lower this ratio.

E. Storage of DNA Samples

The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

IV. Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Homogenization was incomplete	Depending on the type of soil, further vortexing with the flat bed vortex or bead beater equipment may be required. However, it is not recommended to increase the vortex time to longer than 10 minutes at maximum speed. Also, ensure that the maximum recommended soil input is not exceeded, as this may also cause incomplete homogenization.
	An alternative elution buffer was used	It is recommended that the **Elution Buffer** supplied with this kit be used for maximum DNA recovery.
	Lysis Additive was not added to the lysate	Ensure that the provided **Lysis Additive** is added to separate humic acid and increase DNA yield.
	Binding Solution was not added to the lysate	Ensure that the **Binding Solution** is added to the lysate and that it is incubated on ice for 5 minutes prior to spinning down the lysate.
	Ethanol was not added to the lysate	Ensure that an equal amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution II	Ensure that 42 mL of 95 - 100% ethanol is added to the supplied **Wash Solution II** prior to use.
DNA does not perform well in downstream applications	Eluted DNA sample is brown	Ensure that the **Lysis Additive** is added. Also, avoid any contact with the pellet or surface residue when collecting the supernatant during sample preparation.
	Too much soil input was processed	Ensure that the maximum input of soil is not exceeded, as this may also cause incomplete homogenization.
	Lysis Additive was not added to the lysate	Ensure that the provided Lysis Additive is added to the lysate. Also, an incubation can be preformed at 65°C for 10 minutes after addition of the Lysis Additive and prior to vortexing to maximize DNA recovery.

Problem	Possible Cause	Solution and Explanation
DNA does not perform well in downstream applications	DNA was not washed three times with the provided Wash Solutions	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the provided **Wash Solutions**. Salt may interfere with downstream applications, and therefore must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
	PCR reaction conditions need to be optimized	Take steps to optimize the PCR conditions being used, including varying the amount of template, changing the source of *Taq* polymerase, verifying the primer design and adjusting the primer annealing conditions.

V. Related Products

A. Additional RNA Purification Kits

Catalog #	Product Description
BP2800-50	SurePrep™ TrueTotal™ RNA Purification Kit
BP2801-25	SurePrep™ Small RNA Purification Kit
BP2802-50	SurePrep™ RNA/DNA/Protein Purification Kit
BP2803-50	SurePrep™ Urine Exfoliated Cell RNA Purification Kit
BP2804-50	SurePrep™ Urine Bacterial RNA Purification Kit
BP2805-50	SurePrep™ Nuclear Or Cytoplasmic RNA Purification Kit
BP2806-50	SurePrep™ RNA/Protein Purification Kit
BP2807-50	SurePrep™ Leukocyte RNA Purification Kit
BP2809-50	SurePrep™ RNA Cleanup and Concentration Kit
BP2814-25	SurePrep™ Water RNA/DNA Purification Kit
BP2815-50	SurePrep™ Soil DNA Isolation Kit
BP2816-50	SurePrep™ FFPE RNA Isolation Kit
BP2817-50	SurePrep™ Plant/Fungi Total RNA Purification Kit

B. Other Fisher BioReagents Functionally Tested for RNA Research

BP2484-50	Water, Sterile (DEPC-treated) 50mL
BP2484-100	Water, Sterile (DEPC-treated) 100mL
BP561-1	Water, Sterile (RNA Grade) 1L
BP2483-100	EDTA 0.5 M (DEPC-treated) 100mL
BP2483-1	EDTA 0.5 M (DEPC-treated) 1L
BP2483-500	EDTA 0.5 M (DEPC-treated) 500mL
BP2810-50	RiboLadder™ 100b RNA Standard with loading buffers
BP2811-50	RiboLadder™ 1Kb RNA Standard with loading buffers

BP3224-5	Optizyme™ Ribonuclease Inhibitor (Human Placental) 10,000U
BP3224-1	Optizyme™ Ribonuclease Inhibitor (Human Placental) 2,500U
BP3225-5	Optizyme™ Ribonuclease Inhibitor (Porcine) 10,000U
BP3225-1	Optizyme™ Ribonuclease Inhibitor (Porcine) 2,500U
BP3222-5	Optizyme™ Ribonuclease Inhibitor (Recombinant) 10,000U
BP3222-1	Optizyme™ Ribonuclease Inhibitor (Recombinant) 2,500U
BP3226-1	Optizyme™ Recombinant DNase I (RNase-free) 1,000U
BP3226-2	Optizyme™ Recombinant DNase I (RNase-free) 2,000U
BP176-100	2-Mercaptoethanol 100g
BP535-1	Lysozyme, Egg White 1g
BP535-5	Lysozyme, Egg White 5g
BP535-10	Lysozyme, Egg White 10g
BP2476-100	Tris-EDTA, 1X Solution, pH 7.4 100ml
BP2476-500	Tris-EDTA, 1X Solution, pH 7.4 500ml
BP160-100	Agarose, Low EEO, Multipurpose 100g
BP1360-100	Agarose, Low Melting, <1kb RNA 100g
BP1356-100	Agarose, Broad Separation Range for RNA 100g
BP1302-10	Ethidium Bromide, 1% Solution 10ml
BP2900-500	MOPS 10X Solution 500ml
BP2900-1	MOPS 10X Solution 1L

Fisher BioReagents Technical Support

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