

For Research Use Only



## INSTRUCTION

# MANUAL

## Soil DNA Extraction Kit

IB47800 (4 Preparation Sample Kit)

IB47801 (50 Preparation Kit)

IB47802 (100 Preparation Kit)

# Advantages

**Sample:** 250-500 mg of soil

**gDNA Yield:** up to 5 µg

**Format:** beadbeating tubes, PCR inhibitor removal columns and genomic DNA spin columns

**Time:** within 40 minutes

**Elution Volume:** 30-100 µl

**Kit Storage:** dry at room temperature (15-25°C)

## Introduction

The Soil DNA Extraction Kit was designed for rapid isolation of genomic DNA from microorganisms such as bacteria, archaea, fungi, and algae in soil samples. The soil sample is homogenized and disrupted using SL1 lysis buffer combined with ceramic beads. Insoluble particles, proteins and PCR inhibitors such as humic acid are then precipitated with a unique inhibitor removal buffer (SL2). In addition, residual PCR inhibitors remaining in the clear supernatant are further removed by passing through a specialized PCR Inhibitor Removal Column. The flow-through is then mixed with a binding buffer (SL3) and the genomic DNA is bound by the GD Column. The column is then washed and the DNA is eluted with Elution Buffer. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 40 minutes. The purified genomic DNA is ready for use in PCR, restriction enzyme digestion, and sequencing reactions.

## Quality Control

The quality of the Soil DNA Extraction Kit is tested on a lot-to-lot basis by isolating genomic DNA from 250 mg soil samples. Following the purification process, a yield of more than 2 µg of genomic DNA is obtained and the A260/A280 ratio is between 1.7-2.0. The purified genomic DNA is analyzed by electrophoresis.

## Kit Contents

Component	IB47800	IB47801	IB47802
SL1 Buffer <sup>1</sup>	2 ml x 2	50 ml	85 ml
SL2 Buffer	1 ml	15 ml	30 ml
SL3 Buffer	10 ml	45 ml x 2	160 ml
Wash Buffer <sup>2</sup> (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	25 ml (100 ml)
Elution Buffer	1 ml	6 ml	30 ml
Inhibitor Removal Columns	4 pcs	50 pcs	100 pcs
GD Columns	4 pcs	50 pcs	100 pcs
Beadbeating Tubes (Type C)	4 pcs	50 pcs	100 pcs
2 ml Centrifuge Tubes	4 pcs	50 pcs	100 pcs
2 ml Collection Tubes	4 pcs	50 pcs	100 pcs

<sup>1</sup> If precipitates have formed in SL1 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve.

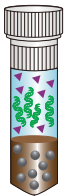
<sup>2</sup> Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

**!** **IMPORTANT BEFORE USE!** During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

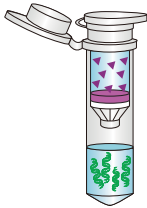
## Quick Protocol Diagram



Soil sample homogenization and lysis with Beadbeating Tube and SL1 Buffer.



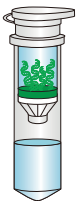
PCR inhibitor removal preparation using SL2 Buffer.



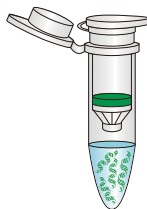
PCR inhibitor removal using the Inhibitor Removal Column.



DNA binding to membrane while contaminants remain suspended.



Wash (removal of contaminants while DNA remains bound to membrane).



Elution of pure genomic DNA which is ready for subsequent reactions.

# Soil DNA Extraction Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

**⚠ IMPORTANT BEFORE USE!** If precipitates have formed in SL1 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

## Additional Requirements

1.5 ml microcentrifuge tubes, standard vortex, absolute ethanol.

## Protocol Procedure

### 1. Sample Lysis

Transfer 250-500 mg of soil to a Beadbeating Tube containing ceramic beads. Add 750 µl of SL1 Buffer then vortex briefly.

**NOTE:** Very dry soil samples can soak up large amounts of SL1 Buffer. In this case, either reduce the soil amount or add additional SL1 Buffer to the Beadbeating Tube. For wet soil samples, after transferring to a Beadbeating Tube, centrifuge at 8,000 x g for 1 minute. Remove as much liquid as possible with a pipette before adding SL1 Buffer. **For frozen soil samples**, incubate the Beadbeating Tube at 70°C for 10 minutes.

Attach the Beadbeating Tubes horizontally to a standard vortex by taping or using an adapter. Vortex at maximum speed for 10 minutes at room temperature. Centrifuge the Beadbeating Tubes at 8,000 x g for 2 minutes at room temperature to eliminate the foam caused by detergents present in SL1 Buffer.

**NOTE:** Preheat the required Elution Buffer (100 µl per sample) to 60°C for DNA elution.

### 2. PCR Inhibitor Removal

Add 150 µl of SL2 Buffer to the Beadbeating Tube and vortex for 5 seconds. Incubate at 0-4°C for 5 minutes. Centrifuge at 8,000 x g for 1 minute at room temperature to precipitate insoluble particles and PCR inhibitors. Place an Inhibitor Removal Column (purple ring) in a 2 ml Centrifuge Tube. Transfer 500-600 µl of clear supernatant from the Beadbeating Tube to the Inhibitor Removal Column. Centrifuge at 16,000 x g for 1 minute at room temperature then discard the column. Save the flow-through in the 2 ml Centrifuge Tube for DNA Binding.

**NOTE:** If a pellet is in the flow-through, transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (not provided).

### 3. DNA Binding

Add 900 µl of SL3 Buffer to the flow-through then mix IMMEDIATELY by shaking vigorously for 5 seconds. Place a GD Column (green ring) in a 2 ml Collection Tube. Transfer 750 µl of sample mixture to the GD Column. Centrifuge at 16,000 x g for 1 minute at room temperature then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. Transfer the remaining sample mixture to the GD Column. Centrifuge at 16,000 x g for 1 minute at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube.

### 4. Wash

Add 400 µl of SL3 Buffer to the GD Column. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Add 600 µl of Wash Buffer (make sure absolute ethanol was added) to the GD Column. Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Add 600 µl of Wash Buffer (make sure absolute ethanol was added) to the GD Column again.

Centrifuge at 16,000 x g for 30 seconds at room temperature. Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. Centrifuge at 16,000 x g for 3 minutes at room temperature to dry the column matrix.

## 5. Elution

Transfer the dry GD Column to a new 1.5 ml microcentrifuge tube. Add 30-100 µl of preheated Elution Buffer<sup>1</sup>, TE<sup>2</sup> or water<sup>3</sup> into the CENTER of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 16,000 x g for 2 minutes at room temperature to elute the purified DNA.

<sup>1</sup> If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 100 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the GD Column matrix and is completely absorbed.

<sup>2</sup> Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed.

<sup>3</sup> If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH<sub>2</sub>O should be fresh as ambient CO<sub>2</sub> can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

# Troubleshooting

## Low Yield

### **Too much starting material.**

Too much soil was added to the Beadbeating Tube. An ample amount of space is required in the Beadbeating Tube to allow the beads to efficiently disrupt the sample.

### **Sample lysis or homogenization was incomplete.**

Horizontally vortex the Beadbeating Tube at the maximum speed using a vortex at room temperature for 10 minutes or using a Disruptor Genie or similar.

### **Incorrect DNA elution.**

Pre-heat the Elution Buffer to 60°C prior to DNA elution. Make sure Elution Buffer is added to the center of the GD Column and is absorbed completely.

### **Inappropriate buffer preparation.**

Add appropriate volume of absolute ethanol (see the bottle label) to the Wash Buffer prior to use. If precipitates have formed in SL1 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve.

## Degraded DNA

### **Mechanical sample disruption is too vigorous.**

Using an alternative lysis method to avoid DNA shearing: After adding SL1 Buffer, vortex the Beadbeating Tube at maximum speed for 5 seconds then incubate the Beadbeating Tube at 70°C for 5 minutes. Repeat these steps 3 times. This lysis method will reduce DNA shearing but may also reduce DNA yield.

## Eluted DNA Does Not Perform Well In Downstream Applications

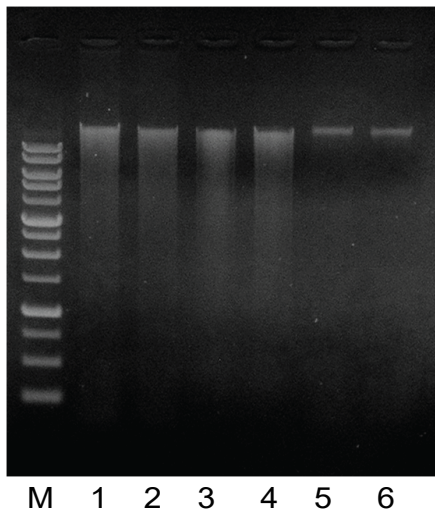
### Residual ethanol contamination.

Following the wash step, dry the GD Column with additional centrifugation at 16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.

### PCR inhibitor contamination.

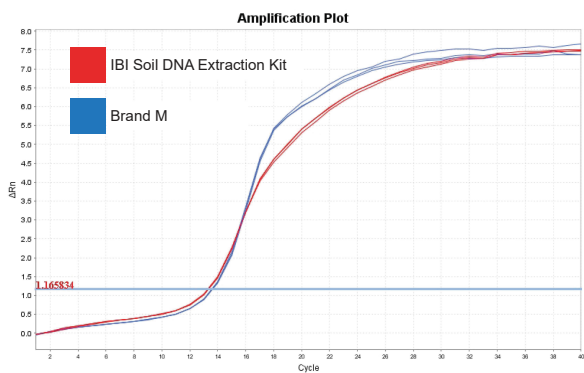
Use diluted DNA (1:10) as a template to reduce the concentration of PCR inhibitors for PCR reactions. DNA can be further purified using the PCR Cleanup Kit to eliminate PCR inhibitors.

## Soil DNA Extraction Kit Functional Test Data

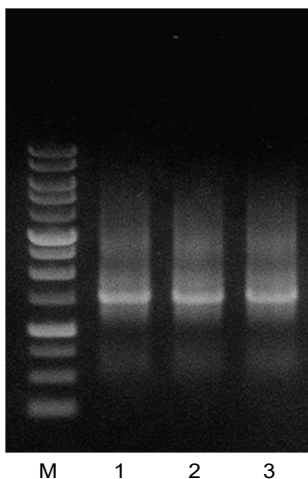


**Figure 1.** Total DNA was purified from 300 mg farm soil samples using three different soil DNA Extraction Kits. 10  $\mu$ l aliquots of purified DNA from 100  $\mu$ l eluates were analyzed on a 0.8% agarose gel. M = 1 Kb DNA Ladder, Lane 1-2: IBI Soil DNA Extraction Kit, Lane 3-4: Brand M, Lane 5-6: Brand E

Product	Yield ( $\mu$ g)	260/280	260/230
IBI Scientific	2.2	1.82	1.89
	2.0	1.83	1.91
Brand M	2.4	1.79	1.20
	2.5	1.84	1.64
Brand E	1.5	1.59	0.50
	1.7	1.55	0.55



**Figure 2.** Total DNA was purified from 300 mg farm soil samples using the IBI Soil DNA Extraction Kit and Brand M kit. Total DNA was eluted with 50  $\mu$ l Elution Buffer. A Real-time PCR assay was performed with 1  $\mu$ l of undiluted eluate (30 ng) as PCR template (3 replications), bacterial 16S rDNA universal primers, and Fast SYBR Green PCR Master Mix using the StepOnePlus™ Real-Time PCR system (Applied Biosystems). The average cycle threshold (Ct) from DNA extracted using the IBI kit is 13.15 compared to 13.66 from the Brand M kit. The lower Ct values indicate a higher number of target nucleic acid in the sample.



**Figure 3.** Total DNA was purified from 300 mg farm soil samples using the IBI Soil DNA Extraction Kit. 2  $\mu$ l of undiluted eluates as template (3 replications) were analyzed by PCR using bacterial 16S rDNA universal primers. The results show that full length bacterial 16S rDNA fragments (1.5 kb) were successfully amplified.

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