Geneaid

Instruction Manual
Ver. 10.21.17
For Research Use Only

Presto™ Soil DNA Extraction Kit

SLD004 (4 Preparation Sample Kit)
SLD050 (50 Preparation Kit)
SLD100 (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)

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Geneaid Biotech Ltd.
Tel: 886 2 26960999 · Fax: 886 2 26960599 · www.geneaid.com · info@geneaid.com

Introduction

The Presto™ 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 using a lysis buffer combined with ceramic beads. Insoluble particles, proteins and PCR inhibitors such as humic acid are then precipitated using a unique inhibitor removal buffer. Residual PCR inhibitors remaining in the clear supernatant are further removed by passing through a specialized PCR inhibitor removal column. Genomic DNA in the sample is then bound by the GD column followed by wash and elution. 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 Presto™ 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 Components

Component	SLD004	SLD050	SLD100
SL1 Buffer[1]	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[2] (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

[1]If precipitates have formed in SL1 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve.

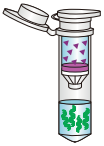
[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

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

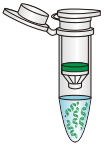
PCR inhibitor removal preparation using SL2 Buffer



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

Presto™ 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 37ºC 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.

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 60ºC for DNA elution.

2. PCR Inhibitor Removal

Add **150 µl of SL2 Buffer to the Beadbeating Tube** and vortex for 5 seconds. Incubate at 0-4ºC 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**[1], TE[2] or water[3] 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.

[1]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.

[2]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.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 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 -20ºC 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 60ºC 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 37ºC 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 70ºC 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 GenepHlow™ PCR Cleanup Kit to eliminate PCR inhibitors.

Presto™ Soil DNA Extraction Kit Functional Test Data

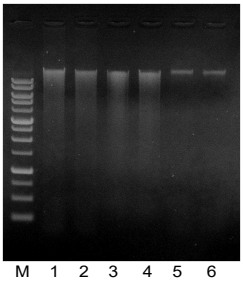


Figure 1. Total DNA was purified from 300 mg farm soil samples using three different soil DNA Extraction Kits. 10 µl aliquots of purified DNA from 100 µl eluates were analyzed on a 0.8% agarose gel. M = Geneaid 1 Kb DNA Ladder, Lane 1-2: Geneaid Presto™ Soil DNA Extraction Kit, Lane 3-4: Brand M, Lane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

Product	Yield (µg)	260/280	260/230
Geneaid	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

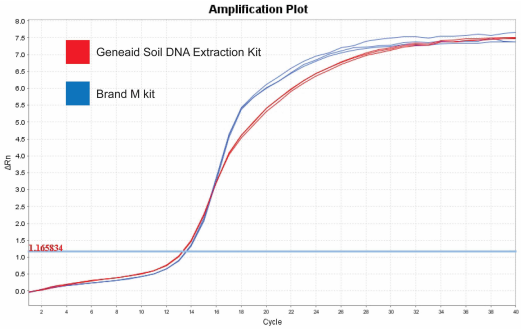


Figure 2. Total DNA was purified from 300 mg farm soil samples using the Geneaid Presto™ Soil DNA Extraction Kit and Brand M kit. Total DNA was eluted with 50 µl Elution Buffer. A Real-time PCR assay was performed with 1 µl of undiluted eluate (30 ng) as PCR template (3 replications), bacterial 16S rDNA 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 Geneaid 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.

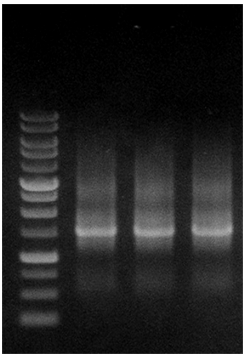


Figure 3. Total DNA was purified from 300 mg farm soil samples using the Geneaid Presto™ Soil DNA Extraction Kit. 2 µl of undiluted eluates as template (3 replications) were analyzed by PCR using bacterial 16S rDNA universal primers. The results show that full length bacterial 16S rDNA fragments (1.5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

Geneaid

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