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Soil Genomic DNA Kit

(Cat# K1411-50, -250; Store at RT)

I. Introduction:

BioVision's Soil Genomic DNA Kit is designed for a rapid and reliable purification of high-quality genomic DNA from various soil samples. Up to 1 gram of soil samples can be processed in <1 hour. The system combines the reversible nucleic acid-binding properties of ezBind matrix with a propriety buffer system to eliminate PCR inhibiting compounds such as humic acid from soil samples. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands on time to allow multiple samples to be processed in parallel.

In this procedure, soil sample is homogenized and then treated in a specially formulated buffer that contains detergent. Hemic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated after a heat-frozen step. DNA is further purified with a DNA spin-column. Two rapid wash steps remove trace contaminants and pure DNA is eluted in water or low ionic strength Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

II. Sample Types:

- Soil

III. Kit Contents:

Components	K1411-50 50 Prep	K1411-250 250 Prep	Part Number
ezBind DNA Columns	50	250	K1411-XX-1
Soil Vial	50	250	K1411-XX-2
DH Reagent	12 mL	60 mL	K1411-XX-3
Buffer LX	40 mL	200 mL	K1411-XX-4
Buffer P2	15 mL	60 mL	K1411-XX-5
Buffer BL*	30 mL	150 mL	K1411-XX-6
DNA Wash Buffer**	10 mL	40 mL	K1411-XX-7
Elution Buffer	20 mL	100 mL	K1411-XX-8
RNase A	160 µL	800 µL	K1411-XX-9

**Buffer BL contains chaotropic salts that may form combustible compound with bleach. Use gloves and protective eye wear when handling this solution. **Add 40 mL (K1411-50) or 160 mL (K1411-250) of absolute ethanol to each DNA Wash Buffer bottle before use.*

IV. User Supplied Reagents and Equipment:

- Microcentrifuge capable of at least 13,000g
- Nuclease-free 1.5 mL or 2 mL microfuge tubes
- Water bath or heating block preset to 65°C and 70°C
- Absolute (96%-100%) ethanol
- Isopropanol (100%)

V. Shipment and Storage:

All the reagents are shipped and stored at room temperature. Under these conditions, DNA has successfully been purified and used for PCR after 12 months of storage. During shipment, or storage in cool ambient conditions, precipitates may form in some buffers. It is possible to dissolve such deposits by incubation the solution at 65°C. The guaranteed shelf life is 12 months from the date of purchase. DO NOT FREEZE!

VI. Reagent Preparation and Storage Conditions:

- Preheat Buffer LX and Elution Buffer at 65°C. Make sure the crystal in Buffer LX is completely dissolved.
- Dilute DNA Wash Buffer with absolute ethanol as follows and store at room temperature. Add 40 mL (K1411-50) or 160 mL of absolute ethanol (K1411-250) to each DNA Wash Buffer bottle before use.

VII. Soil Genomic DNA Purification Protocol:

1. Add 0.25-0.5 g soil sample to a Soil Vial. Vortex briefly for 5 seconds. Add 70 µL **Buffer LX**. Vortex at maximum speed for 5 minutes till the sample is thoroughly homogenized.
2. Incubate at 70°C for 10 min, Mix sample twice during incubation by vortexing the tube. Optional: for isolation of DNA from gram positive bacteria, do a second incubation at 95 °C for 2 minutes. Spin the sample at 10,000 x g for 1 min. Transfer 500 µL of clear lysate to a 2 mL tube. *Note: Make sure the soil vial rotates freely in your centrifuge without rubbing. Centrifuge tubes at 10,000g for 30 seconds at room temperature. CAUTION: Be sure not to exceed 10,000g.*
3. Add 250 µL **Buffer P2**, mix thoroughly by vortexing for 30 seconds. Incubate the sample on ice for 5 minutes.
4. Centrifuge the sample at 10,000g for 2 minutes. Carefully transfer around 600 µL of supernatant, avoiding the pellet, to a 1.5mL microfuge tube.
5. **Add 250 µL of Buffer DH**, mix well by vortexing for 5 seconds and incubate on ice for 5 minutes.
6. Centrifuge at 10,000g for 2 minutes at room temperature.
7. Transfer around 700 µL supernatant, avoid pellet, to a clean vial and add 575 µL of **Buffer BL** and **100 µL isopropanol**. Mix well by vortexing for 5 seconds.
8. Transfer 700 µL of the sample to a **mini column** and spin at 10,000g for 30 seconds. Discard the flow through and reuse the collection tube. Process the remaining sample as described.
9. Add 700 µL of **DNA Wash Buffer** (add ethanol prior to use) to the column and spin at 10,000g for 30 seconds. Discard the flow through and put the column back to the collection tube.
10. Centrifuge the empty column at maximum speed for 2 min. Transfer the column to a 1.5 mL tube.
11. Add 50 µL of **Elution Buffer** directly onto the center of the matrix and incubate at 65°C for 5 minutes.

12. Centrifuge at 10,000g for 1 min to elute DNA. *Optional: Re apply the eluent to the column and spin at 10,000g for 1 min to for a second elution.*

VIII. Related Products:

Product Name	Cat. No.	Size
Bacterial Genomic DNA Isolation Kit	K309-100	100 Isolations
Bacterial Genomic DNA Mini Kit	K1388-100, -300	100 Preps, 300 Preps
Bacterial Genomic DNA Mini Kit (w/Lysozyme)	K1389-100, -300	100 Preps, 300 Preps
Concert™ DNA Extraction Kit	K1390-50, -100, -300	50 Preps, 100 Preps, 300 Preps
Genomic DNA Micro Kit	K1385-100, -300	100 Preps, 300 Preps
Genomic DNA Isolation Kit (Cells/Tissues)	K281-50	50 Assays
Genomic DNA Mini Kit (Blood/Cells)	K1380-100, -300	100 Preps, 300 Preps
Genomic DNA Maxi Kit (Blood/Cells)	K1381-10, -25	10 Preps, 25 Preps
Macro Blood DNA Kit	K1392-3, -1000	100 ml, 1000 ml
Macro Blood DNA Plus Kit	K1393-1000	1000 ml
Macro Bacterial DNA Kit	K1394-3, -1000	100 ml, 1000 ml
Macro Bacterial DNA Plus Kit	K1395-1000	1000 ml
Macro Tissue DNA Kit	K1396-3, -1000	100 ml, 1000 ml
Macro Tissue DNA Plus Kit	K1397-1000	1000 ml
Macro Cell DNA Kit	K1398-3, -1000	100 ml, 1000 ml
Macro Cell DNA Plus Kit	K1399-1000	1000 ml
Mammalian Cell Genomic DNA Isolation Kit	K967-100	100 Isolations
Mitochondrial DNA Isolation Kit	K280-50	50 Assays
Plant Genomic DNA Mini Kit	K1383-100	100 Preps
Plant Genomic DNA Maxi Kit	K1384-10, -25	10 Preps, 25 Preps
Plant Tissue Genomic DNA Isolation Kit	K316-100	100 Isolations
Swab Genomic DNA Kit	K1386-100, -300	100 Preps, 300 Preps
Tissue Genomic DNA Mini Kit	K1382-50, -100, -300	50 Preps, 100 Preps, 300 Preps
Triadzol™ Plant DNA Reagent	M1391-100, -200	100 ml, 200 ml
Yeast Genomic DNA Mini Kit	K1387-100, -300	100 Preps, 300 Preps
96 Well Blood Genomic DNA Kit	K1406-2, -4	2 Plates, 4 Plates
96 Well Plant Genomic DNA Kit	K1407-2, -4	2 Plates, 4 Plates
96 Well Plant Genomic DNA Binding Plate	M1209-5	5 Plates
Whole Blood DNA Isolation Kit	K528-100	100 Isolations

IX. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
$A_{260/230}$ ratio is low	• inefficient elimination of inhibitory compounds • Salt contamination • DNA Wash Buffer prepared with lower percentage ethanol	• Repeat the DNA isolation with a new sample be sure to mix the sample with DH reagent thoroughly and extract the sample with DH reagent twice. • Repeat the DNA isolation with a new sample. Make sure the column is dried before elution. Wash the column with extra DNA Wash Buffer. • Prepare DNA Wash Buffer with 96- 100% ethanol.
$A_{260/280}$ ratio is high	• High RNA contamination	• Be sure to treat the sample with RNase A.
Low DNA yield or no DNA eluted	• Sample stored incorrectly • Poor homogenization of sample • Incorrect Buffer BL was added before loading to the column • DNA washed off.	• Repeat the DNA isolation with a new sample be sure to mix the sample with Buffer LX and glass beads. • Use long beads beating time to make sure the sample are fully homogenized and cells are lysed. • Repeat the DNA isolation with a new sample • Dilute DNA Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use.
Problems in downstream applications	• Ethanol residue in the elute	• Be sure to completely dry the column before elution
Little or no supernatant after initial centrifuge step	• insufficient centrifugal force	• Check the centrifugal force and increase the centrifugal time if necessary
sample cannot pass through the column	• Clogging column	• Check the centrifugal force and increase the time of centrifugation

FOR RESEARCH USE ONLY! Not to be used on humans.