E.Z.N.A.® Soil DNA Kit

Revision No: 1.3

omega BIO-TEK

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	D5625-00	D5625-01	D5625-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
Disruptor Tubes	5	50	200
SLX-Mlus Buffer	6 mL	60 mL	220 mL
DS Buffer	0.6 mL	6 mL	22 mL
P2 Buffer	3 mL	25 mL	60 mL
XP1 Buffer	4 mL	40 mL	160 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	2 mL	20 mL	3 x 25 mL
Elution Buffer	3 mL	30 mL	120 mL
cHTR Reagent	1.2 mL	12 mL	45 mL

Supplied by user:

- Tabletop microcentrifuge capable of 13,000*g*
- Centrifuge with adaptor for 15 mL centrifuge tubes (for 250-1000 mg Soil Protocol)
- Incubator capable of 70°C
- Vortexer
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- 15 mL centrifuge tubes (for 250-1000 mg Soil Protocol)
- Ice bucket
- 100% ethanol
- 100% isopropanol

Before starting:

- Prepare HBC Buffer and DNA Wash Buffer according to the directions on the bottles.
- Prepare an ice bucket.
- Chill P2 Buffer.
- Set incubator to 70°C.
- Heat Elution Buffer to 70°C.

DNA Extraction and Purification from 100–250 mg Soil

LYSE

1. Add 100-250 mg soil sample and 725 µL SLX-Mlus Buffer to a Disruptor Tube. Vortex at maximum speed for 3-5 minutes to lyse samples. Centrifuge at 500*g* for 5 seconds to remove drops of liquid from the lid.
2. Add 72 µL DS Buffer. Vortex to mix thoroughly. Incubate at 70°C for 10 minutes. Briefly vortex the tube once during incubation. Centrifuge at 10,000*g* for 5 minutes at room temperature.

INHIBITOR REMOVAL

3. Transfer 400 µL supernatant into a new 1.5 mL microcentrifuge tube (not provided). Add 135 µL chilled P2 Buffer and 200 µL cHTR Reagent that has been completely resuspended. Vortex to mix thoroughly. Centrifuge at maximum speed for 1 minute.
4. Transfer cleared supernatant to a new 1.5 mL microcentrifuge tube. If supernatant still has a dark color from the soil, add 200 µL cHTR Reagent, vortex to mix thoroughly, and centrifuge at maximum speed for 1 minute. Transfer cleared supernatant to a new microcentrifuge tube.

   Note: This will require additional cHTR Reagent (Cat# CHTR-50) to be purchased separately.

BIND

5. Add an equal volume of XP1 Buffer. Vortex to mix thoroughly.
6. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube. Transfer up to 700 µL sample from Step 5 to the HiBind® DNA Mini Column. Centrifuge at 10,000*g* for 1 minute at room temperature. Discard the filtrate and reuse the Collection Tube.
7. Using the same Collection Tube, repeat Step 6 until all the lysate has passed through the HiBind® DNA Mini Column.

WASH

8. Add 500 µL HBC Buffer diluted with 100% isopropanol. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and Collection Tube.
9. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube. Add 700 µL DNA Wash Buffer diluted with 100% ethanol. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the Collection Tube.

WASH

10. Using the same Collection Tube, repeat Step 9 for a second DNA Wash Buffer wash step.
11. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes at room temperature. This step is critical in removing residual ethanol that may interfere with downstream applications.

ELUTE

12. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 50-100 µL Elution Buffer heated to 70°C directly onto the center of HiBind® matrix. Let sit at room temperature for 1-2 minutes. Centrifuge at maximum speed for 1 minute.
13. Take the filtrate from Step 12 and place onto the center of the same HiBind® DNA Mini Column used in the procedure. Let sit at room temperature for 1 minute. Centrifuge at maximum speed for 1 minute.
14. Store eluted DNA at -20°C.

DNA Extraction and Purification from 250–1,000 mg Soil

LYSE

1. Transfer the glass beads from a Disruptor Tube to a 15 mL centrifuge tube. Add 0.25-1 g soil sample and 1 mL SLX-Mlus Buffer. Vortex at maximum speed for 3-5 minutes to lyse samples.
2. Add 100 µL DS Buffer. Vortex to mix thoroughly. Incubate at 70°C for 10 minutes. Briefly vortex the tube once during incubation. Centrifuge at 3,000 rpm for 3 minutes at room temperature.

INHIBITOR REMOVAL

3. Transfer 800 µL supernatant into a new 2 mL microcentrifuge tube (not provided). Add 270 µL chilled P2 Buffer. Vortex to mix thoroughly. Let sit on ice for 5 minutes. Centrifuge at maximum speed for 5 minutes.
4. Carefully transfer the supernatant to a new 2 mL microcentrifuge tube. Add 0.7 volumes 100% isopropanol. Mix thoroughly by inverting tube for 20-30 times. If the soil contains very low DNA, incubate the sample at -20°C for 1 hour. Centrifuge at maximum speed for 10 minutes. Carefully aspirate and discard the supernatant. Do not disturb the DNA pellet.
5. Invert the tube on absorbent paper for 1 minute to drain the liquid. Add 200 µL Elution Buffer. Vortex for 10 seconds. Incubate at 70°C for 10-20 minutes to dissolve the DNA pellet.
6. Add 100 µL cHTR Reagent that has been completely resuspended. Vortex to mix thoroughly. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 2 minutes.
7. Transfer the cleared supernatant to a new 2 mL microcentrifuge tube. If supernatant still has a dark color from the soil, repeat Step 6 for a second cHTR Reagent step. This will require additional cHTR Reagent (Cat# CHTR-50) to be purchased separately.

BIND

8. Add an equal volume of XP1 Buffer. Vortex to mix thoroughly.
9. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube. Transfer the sample from Step 8 to the HiBind® DNA Mini Column. Centrifuge at 10,000*g* for 1 minute at room temperature. Discard the filtrate and reuse the Collection Tube.

WASH

10. Add 500 µL HBC Buffer diluted with 100% isopropanol. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and the Collection Tube.
11. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube. Add 700 µL DNA Wash Buffer diluted with 100% ethanol. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the Collection Tube.
12. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes at room temperature. This step is critical in removing residual ethanol that may interfere with downstream applications.

ELUTE

13. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 50-100 µL Elution Buffer heated to 70°C directly onto the center of HiBind® matrix. Let sit at room temperature for 1-2 minutes. Centrifuge at maximum speed for 1 minute.
14. Take the filtrate from Step 13 and place onto the center of the same HiBind® DNA Mini Column used in the procedure. Let sit at room temperature for 1 minute. Centrifuge at maximum speed for 1 minute.
15. Store eluted DNA at -20°C.