E.Z.N.A.® Stool DNA Kit

Revision No: 1.1

Quick Guide

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

Product	D4015-00	D4015-01	D4015-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
SLX-Mlus Buffer	12 mL	100 mL	400 mL
DS Buffer	1.2 mL	10 mL	40 mL
SP2 Buffer	5 mL	35 mL	125 mL
BL Buffer	5 mL	35 mL	125 mL
VHB Buffer	2.2 mL	15 mL	66 mL
DNA Wash Buffer	2 mL	20 mL	3 x 20 mL
Elution Buffer	5 mL	30 mL	100 mL
Glass Beads X	1.2 g	12 g	45 g
cHTR Reagent	1.2 mL	12 mL	50 mL
Proteinase K Solution	150 µL	1.5 mL	6 mL

Supplied by user:

- Microcentrifuge capable of 13,000 x *g*
- Centrifuge with adaptor for 15 mL centrifuge tubes capable of 4,000 x *g*
- Incubators, heat blocks, or water baths capable of 65°C and 70°C
- Vortexer
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- 15 mL centrifuge tubes
- Ice bucket
- 100% ethanol
- Optional: RNase A (20 mg/mL) and incubator capable of 37°C
- Optional: Incubator or heat block capable of 95°C

Before starting:

- Prepare VHB Buffer and DNA Wash Buffer according to the directions on the bottles.
- Prepare an ice bucket.
- Set an incubator, heat block, or water bath to 70°C.
- Heat Elution Buffer to 65°C.
- Optional: for RNase digestion, set an incubator to 37°C.
- Optional: for gram-positive bacteria, set an incubator to 95°C.

DNA Extraction and Purification from Stool for Pathogen Detection

LYSE

1. Add up to 200 mg or 200 µL stool sample in a 2 mL microcentrifuge tube (not provided) containing 200 mg Glass Beads X. Place the tube on ice. If the sample is frozen, do not thaw until the SLX-Mlus Buffer is added into the tube.
2. Add 540 µL SLX-Mlus Buffer. Vortex at maximum speed for 10 minutes or until the sample is thoroughly homogenized.
3. Add 60 µL DS Buffer and 20 µL Proteinase K Solution. Vortex or pipet up and down to mix thoroughly. Incubate at 70°C for 10 minutes (13 minutes if frozen). Vortex the sample twice during incubation. For isolation of DNA from gram-positive bacteria, do a second incubation at 95°C for 5 minutes.

INHIBITOR REMOVAL

4. Add 200 µL SP2 Buffer. Vortex at maximum speed for 30 seconds. Let sit on ice for 5 minutes. Centrifuge at maximum speed (≥13,000 x *g*) for 5 minutes.
5. Carefully aspirate 400 µL supernatant to a new 1.5 mL microcentrifuge tube (not provided). Do not disturb the pellet or transfer any debris.
6. Add 200 µL cHTR Reagent that has been completely resuspended. Vortex at maximum speed for 10 seconds. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 2 minutes.
7. Transfer 250 µL supernatant to a new 1.5 mL microcentrifuge tube (not provided).

Optional: If RNA-free DNA is required, add 10 µL RNase A (not provided). Vortex to mix thoroughly. Incubate at 37°C for 3 minutes. Continue to Step 8.

BIND

8. Add 250 µL BL Buffer and 250 µL 100% ethanol. Vortex at maximum speed for 10 seconds.
9. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube. Transfer the entire sample from Step 8, including any precipitates that may have formed, to the HiBind® DNA Mini Column. Centrifuge at maximum speed for 1 minute. Discard the filtrate and the collection tube.

WASH

10. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube. Add 500 µL VHB Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 30 seconds. Discard the filtrate and reuse the collection tube.
11. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse collection tube.
12. Repeat Step 11 for a second DNA Wash Buffer wash step.
13. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

ELUTE

14. Transfer the column into a clean 1.5 mL microcentrifuge tube. Add 100-200 µL Elution Buffer heated to 65°C. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute.
15. Store DNA at -20°C.

DNA Extraction and Purification from Stool for Human DNA Detection

LYSE

1. Add up to 200 mg or 200 µL stool sample in a 15 mL centrifuge tube (not provided) and place the tube on ice. Add 1.6 mL SLX-Mlus Buffer. Vortex at maximum speed for 1 minute or until the stool sample is completely homogenized. If the sample is frozen, do not thaw until the SLX-Mlus Buffer is added into the tube.
2. Add 180 µL DS Buffer. Invert 5 times to mix. Centrifuge at maximum speed (≥4,000 x *g*) for 3 minutes.
3. Transfer 1.5 mL supernatant into a clean 15 mL centrifuge tube.

INHIBITOR REMOVAL

4. Add 600 µL SP2 Buffer. Vortex at maximum speed for 10 seconds. Let sit on ice for 5 minutes. Centrifuge at maximum speed (≥4,000 x *g*) for 3 minutes.
5. Transfer 600 µL cleared supernatant to a new 2 mL microcentrifuge tube (not provided). Add 200 µL cHTR Reagent. Vortex at maximum speed for 10 seconds. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 2 minutes.
6. Transfer 600 µL supernatant into a new 2 mL microcentrifuge tube. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

BIND

7. Add 600 µL BL Buffer. Vortex at maximum speed for 10 seconds. Incubate at 70°C for 10 minutes. Vortex the sample twice during incubation. Centrifuge briefly to remove any liquid drops from the tube lid.
8. Add 600 µL 100% ethanol. Vortex at maximum speed for 10 seconds. Centrifuge briefly to remove any liquid drops from the tube lid.
9. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube. Transfer 600 µL sample from Step 8, including any precipitates that may have formed, to the HiBind® DNA Mini Column. Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse collection tube.
10. Repeat Step 9 until all of the sample has been transferred to the HiBind® DNA Mini Column.
11. Proceed to Step 10 of the DNA EXTRACTION AND PURIFICATION FROM STOOL FOR PATHOGEN DETECTION protocol above.