SPINeasy™ Host Depletion Microbial DNA Kit

Cat. No.: 116545050 (50 PREPS) / 116545000 (5 PREPS)

Quick-Start Protocol

Revision 2.0 Aug 2023

Scan QR code for more information from instruction manual

Notes before starting:

- Upon receipt, store **Host Depletion Enzyme HDE and Microbial Selection Buffer** at 4 °C.
- Add 50 mL (5 mL for sample kit) of absolute ethanol to Wash Buffer HD2 and mark the bottle.
- Prepare Host Depletion Enzyme HDE solution: Spin down components briefly and add 120 µL of **HDE Reconstitution Buffer** to the entire content in the vial of **Host Depletion Enzyme HDE.** Mix well to dissolve and store reconstituted solution at -20 °C.

Host Cell Lysis

1. In a 2 mL microcentrifuge tube, add 200 µL of sample to 1 mL of **Host Lysis Buffer.**
2. Vortex at for 10 seconds.
3. Incubate for 15 mins at room temperature with constant end-over-end tube rotation.
4. Vortex at for 10 seconds and centrifuge at 10,000 x g for 5 mins.
5. Carefully aspirate and discard supernatant without disturbing the pellet.

Host DNA Depletion

6. Resuspend pellet in 100 µL of **Host Depletion Buffer.**
7. Add 1 µL of Host Depletion Enzyme HDE solution* and vortex briefly to mix.
   *Prepare Host Depletion Enzyme HDE solution according to instructions in "Notes before starting"
8. Incubate at 37 °C for 10 mins.
9. Add 200 µL of **Microbial Selection Buffer** and vortex briefly to mix.
10. Incubate at 37 °C for 30 mins.
11. Vortex for 10 seconds and spin down briefly to collect contents at the bottom of the tube.

Microbial Cell Lysis

12. Add 700 µL of **Microbial Lysis Buffer.**
13. Mix by pipetting up and down several times and transfer all the mixture to a vial of **Lysing Matrix E.**
14. Homogenize in a FastPrep Instrument for 10 seconds at speed setting of 6.0 m/s.
    If a FastPrep Instrument is not available, vortex samples at the maximum speed for 1 – 5 mins.
15. Centrifuge at 14,000 x g for 10 mins.

Microbial DNA Purification

16. Add 200 µL of **Equilibration Buffer** to the **Column HD with collection tube** membrane.Wait for 1min and centrifuge at 14,000 x g for 1 min, discard flow through and reuse collection tube.
17. Transfer 750 µL of the supernatant to a Column HD with collection tube.
18. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
19. Add 500 µL of **Wash Buffer HD1.** Centrifuge at 14,000 x g for 1 min, discard flow through and reuse collection tube.
20. Add 750 µL of **Wash Buffer HD2.** Centrifuge at 14,000 x g for 1 min, discard flow through and reuse collection tube.
21. Centrifuge at 14,000 x g for an additional 1 min to dry column.
22. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
23. Add 50 µL of **Elution Buffer HD** to the center of the membrane. Incubate at room temperature for 5 mins.
24. Centrifuge at 8,000 x g for 2 mins to elute microbial DNA. Store DNA at - 20 °C.

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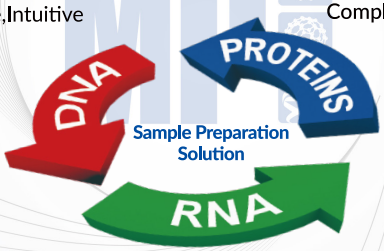

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