

SPINeasy Host Depletion Microbial DNA Kit

Spin Column PCR Purification and Gel Extraction

Size: 50 & 5 preps
Storage: 15-25 °C
Cat. No.: 116545050 (50 PREPS)
116545000 (5 PREPS)
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1. Introduction to SPINeasy Host Depletion Microbial DNA Kit

Host DNA contamination impedes the molecular analyses of microbiomes in host samples, such as bodily fluids and swabs. The SPINeasy Host Depletion Microbial DNA Kit provides an easy-to-use workflow to isolate microbial DNA from samples containing high amounts of host DNA. This background reduction of host DNA is achieved through selective lysis of host cells with our specially formulated Host Lysis Buffer. Host DNA, which has been released in the solution, is then removed from the supernatant through centrifugation. A further host depletion treatment provides enzymatic degradation of any remaining host DNA, as well as DNA from dead microbial cells. Finally, the remaining intact cells are lysed through a combination of specially formulated buffers and highly efficient homogenization using FastPrep® Instruments from MP Biomedicals. Microbial DNA is purified using a convenient silica-membrane spin-column technology workflow and ready for downstream molecular applications.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini spin column
Vacuum manifold	Yes
Sample	Host-microbial mixed sample
Sample amount	200 µL
Elution volume	50 µL

2. Kit Components and User Supplied Materials

2.1 SPINeasy Host Depletion Microbial DNA Kit Component

50 PREPS (Cat.No.: 116545050)		
Components	Package	Cat. No.
Equilibration Buffer	12 mL	116547059
Lysing Matrix E	50 ea	116914050
Host Lysis Buffer	60 mL	116545051
Host Depletion Buffer	6 mL	116545052
Host Depletion Enzyme HDE	1 vial	116545053
HDE Reconstitution Buffer	200 µL	116545054
Microbial Selection Buffer	12 mL	116545055
Microbial Lysis Buffer	40 mL	116545056
Wash Buffer HD1	30 mL	116545057
Wash Buffer HD2	6 mL	116545058
Elution Buffer HD	6 mL	116545059
Column HD with collection tubes	50 ea	116545060
Quick-Start Protocol	1 ea	-
Instruction Manual	Available www.mpbio.com	
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500)
- Vortex mixer with adapter
- Microcentrifuge capable of at least 14,000 g
- Tube rotator
- Absolute ethanol (50 mL)
- 1.5 mL microcentrifuge tubes
- 2.0 mL microcentrifuge tubes
- Heat block, water bath or incubator set at 37°C
- Optional: 1M DTT (for extraction from saliva samples)

- Single-channel pipettors (1 μ L-1000 μ L)
- Nuclease-free, aerosol-preventive tips
- Biohazard disposal containers
- Personal Protective Equipment

3. Storage and Kit Stability

Upon receipt, store Host Depletion Enzyme HDE and Microbial Selection Buffer at 4°C. Store reconstituted Host Depletion Enzyme HDE solution at -20°C. All other components and reagents of the SPINeasy Host Depletion Microbial DNA Kit can be stored at room temperature (15-25°C) until the expiration date printed on the kit label. For extended storage or storage in dry condition (humidity < 40%), store the columns at 2-8°C to maintain performance.

4. Important Consideration Before Use

- Add 50 mL (5 mL for sample kit) of absolute ethanol to Wash Buffer HD2 and mark the bottle.
- Reconstitute Host Depletion Enzyme according to instructions in Section 6 (No.1).
- Prepare a 2 mL microcentrifuge tube per prep for sample preparation.
- Prepare a 1.5 mL microcentrifuge tube per prep for DNA elution.
- For saliva sample: Add a final concentration of 5 mM DTT (user-supplied) and mix.
- Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if 14,000 g is not feasible.

5. Safety Precautions

Host Lysis Buffer, Microbial Lysis Buffer, and Wash Buffer HD1 contain components that can be harmful if swallowed and may cause irritation when in contact with skin and eyes. To prevent accidental ingestion, do not eat, drink, or smoke when using this product. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

6. Protocol

1. Preparation of Host Depletion Enzyme Solution

Briefly spin down the vials of **HDE Reconstitution Buffer** and **Host Depletion Enzyme HDE** provided to collect contents at the bottom of the tubes. Add **120 µL HDE Reconstitution Buffer** to the vial of **Host Depletion Enzyme HDE** and mix well to dissolve.

Important: Store *Host Depletion Enzyme HDE solution* at **-20 °C** after reconstitution. Solution will not freeze.

2. Column HD Preparation

- Add **200 µL Equilibration Buffer** to the **Column HD** membranes to ensure its performance.
- Wait at least **1 min** and centrifuge for **10 sec @ maximum speed**. Transfer the **Column HD** into a new collection tube (provided).

3. Host lysis

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

4. Host Depletion and Microbial Selection

- Resuspend pellet in **100 µL Host Depletion Buffer**.
- Add **1 µL Host Depletion Enzyme HDE solution** and vortex briefly to mix.
Note: Prepare *Host Depletion Enzyme HDE solution* according to instructions in Section 6 (No.1).
- Incubate for **10 min @ 37 °C**.
- Add **200 µL Microbial Selection Buffer** and vortex briefly to mix.
- Incubate for **30 min @ 37 °C**.

- Vortex for **10 sec** and spin down briefly to collect contents at the bottom of the tube.

5. Microbial lysis

- Add **700 µL Microbial Lysis Buffer**.
- Mix by pipetting up and down several times and transfer all the mixture to a vial of **Lysing Matrix E**.
- Homogenize in a FastPrep Instrument for **10 sec** at speed setting of **6.0 m/s**.
- Alternatively, vortex samples for **1 - 5 min @ maximum speed** if a FastPrep Instrument is not available. Homogenization speed and duration may be adjusted to achieve optimal lysis for each sample type.
- Centrifuge for **10 min @ 14,000 g**.
Note: Centrifuge at the maximum speed for all steps if 14,000 g is not feasible.
- Transfer **750 µL** of the supernatant to a Column HD with collection tube.
- Centrifuge for **1 min @ 14,000 g**. Discard flow through and reuse collection tube.

6. Wash

- Add **500 µL Wash Buffer HD1** to the column.
- Centrifuge for **1 min @ 14,000 g**. Discard flow through and reuse collection tube.
- Add **750 µL Wash Buffer HD2** to the column.
- Centrifuge for **1 min @ 14,000 g**. Discard flow through and reuse collection tube.
- Centrifuge for an additional **1 min @ 14,000 g** to dry the column.

7. Elution

- Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
- Add **50 µL Elution Buffer HD** to the center of the membrane.
- Incubate for **5 min @ room temperature**.
- Centrifuge for **2 min @ 8,000 g**.
- Eluted microbial DNA will be collected in the microcentrifuge tube and ready for downstream applications. Store DNA at **-20 °C**.

7. Data

The following are PCR results of DNA extraction from saliva samples using SPINeasy Host Depletion Microbial DNA Kit, showing effective host DNA depletion and microbial DNA recovery. Both host DNA depletion and bacteria DNA recovery are estimated to be higher than 90% for these samples, using qPCR analyses.

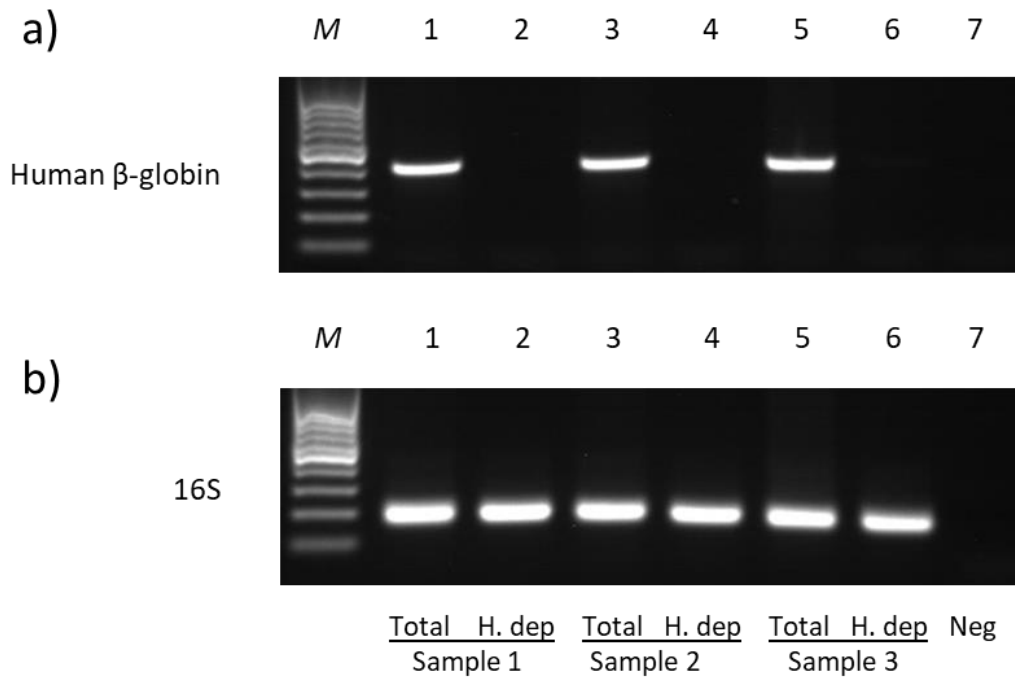


Figure 1: PCR of DNA extracted from saliva samples using SPINeasy Host Depletion Microbial DNA Kit. a) PCR detection of host DNA using human β -globin primers. b) PCR detection of bacterial DNA using 16S primers. M: DNA marker; Lane 1, 3, 5: Total DNA extracted without performing host depletion steps; Lane 2, 4, 6: Host depleted (H. dep) DNA extracted using SPINeasy Host Depletion Microbial DNA Kit; Lane 7: PCR negative control.

8. Troubleshooting

This guide may be useful in solving any problems that may arise. For further assistance, please contact our technical support team at apac-techsupport@mpbio.com

Problem	Possible Cause	Recommendation
Poor host DNA depletion	Insufficient mixing of sample and Host Lysis Buffer	Increase vortexing time if necessary.
	Improper Host Depletion Enzyme HDE reconstitution.	Ensure that Host Depletion Enzyme HDE has been reconstituted according to instructions. Store Host Depletion Enzyme HDE solution at -20 °C after reconstitution.
Poor/no microbial DNA recovered	No intact microbial cells in the sample	Use fresh samples and avoid freezing. Ensure sample storage conditions will not cause microbial lysis.
	Lost bacteria pellet	Avoid disturbing the pellet when aspirating host lysate supernatant.
	Decreased activity of Microbial Selection Buffer	Store Microbial Selection Buffer at 4 °C. Do not freeze.
	Missed addition of ethanol to Wash Buffer HD2	Ensure that ethanol has been added to Wash Buffer HD2.
	Poor elution	Ensure that Elution Buffer HD is added to the center of the column membrane.
DNA does not perform well in downstream reactions	Low starting amounts of microorganism	Pool several host-depleted microbial lysate supernatants and load onto a single column. Note that the DNA concentration in the eluent may not be detectable by NanoDrop™, but can still be used in downstream molecular applications.
	Little or no DNA eluted.	Refer to Poor/no microbial DNA recovered section.
	Ethanol carry-over	Ensure that the flow-through is discarded after washing with Wash Buffer HD2 and the column is centrifuged an additional time to dry the membrane.

9. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices to diagnose, cure, mitigate, treat, or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery.

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