SPINeasy™ DNA Kit for Microbiome

Cat. No.: 116553050 (50 PREPS) / 116553000 (5 PREPS)

Quick-Start Protocol

Revision Oct 2023

The kit allow efficient and unbiased lysis of microbes including gram positive/negative bacteria, fungi, protozoans, and viruses found in various samples including soil, swabs, body fluids, milk etc.

Scan QR code for more information from instruction manual

Notes before starting:

- **If Buffer MB1** has precipitated, heat at **37 °C** until precipitate dissolves.
- Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if **15,000 *g*** is not feasible.
- Vortex the samples at **2,500-3,000 rpm** for **10 min** if a **FastPrep®** instrument is unavailable. Secure samples on the vortex through an adaptor to ensure homogenization.
- For fast processing, the protocol is compatible with vacuum manifold.

Column Preparation

***Optional:* Prepare column to ensure its performance**

*Pipette **200 µL Equilibration Buffer** into **Column MB**. Incubate for **1 min** at room temperature and centrifuge for **30 sec @14,000 g. Discard flow-through and reuse collection tube.***

Lyse & Remove inhibitor

DNA isolation protocol:

1. **Add 10^7~10^{10} cfu bacterial sample to Lysing Matrix E tube.**
   ***Note:** Refer to next page for recommended amounts and summary of sample preparation.*
2. Add **800 µL Buffer MB1** and **25-40 µL RNase A Solution,** invert the tubes several times to mix the lysing matrix and buffer. Homogenize in a FastPrep® instrument for **45 sec** twice at speed setting of **5 m/s** with a **5 minutes** interval. Centrifuge for **2 min @ ≥15,000 *g*.**
3. Transfer all supernatant into 2 mL centrifuge tube (self-provided). Add **300 µL Buffer MB2**, invert and mix 5 times. Centrifuge for **2 min @ ≥15,000 *g*.**
4. Transfer the supernatant (~750 µL) to a clean 2 mL centrifuge tube (self-provided). Add **750 µL Buffer MB3,** invert and mix twice.

Binding

Wash

Microcentrifuge Method

5. Transfer ~**750 µL** mixture to Column MB.
6. Centrifuge for **1 min @ ≥15,000 *g*** and discard the flow-through. Repeat the process until all the lysate has passed through.
7. Add **500 µL Buffer MB4** to the center of the column, centrifuge for **1 min @ 15,000 *g***, discard the flow-through and place the column back into the same 2 mL collection tube.
8. Add **700 µL Buffer MB5** to the center of the column and centrifuge for **1 min @ 15,000 *g*** and discard the flow-through.

Vacuum Manifold Method

5. Insert the Column MB into the vacuum manifold's luer connectors. Load ~**750 µL** mixture into the Column MB and apply vacuum.
6. Repeat until all the mixture has been loaded. Switch off the vacuum source to avoid membrane over drying.
7. Add **500 µL Buffer MB4** to the center of the column and apply vacuum. Switch off the vacuum source.
8. Add **700 µL Buffer MB5** by running the pipette tip along the wall of the column and apply vacuum. Switch off the vacuum source.

Elute

9. Without addition of any liquid, centrifuge for **2 min @ 15,000 *g*** to dry the column.
10. Discard collection tube and place the Column MB into a new **1.5 mL Collection Tube** (provided).
11. Add **45-50 µL Buffer MB6** to the middle of the membrane column **slowly**. Centrifuge for **1 min @ 15,000 *g***. Reload the elute or add **45-50 µL fresh Buffer MB6** into the column Centrifuge for **1 min** at maximal speed.
    ***Note:** For maximal yield, the elution volume can be expanded to **200 µL**.*

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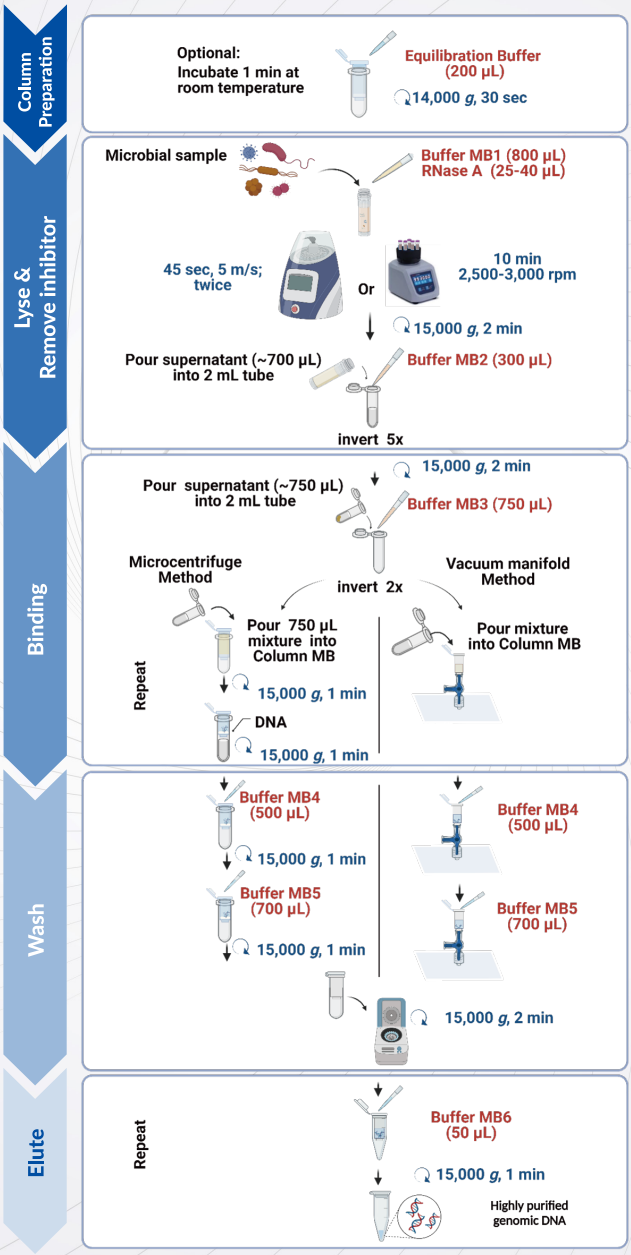
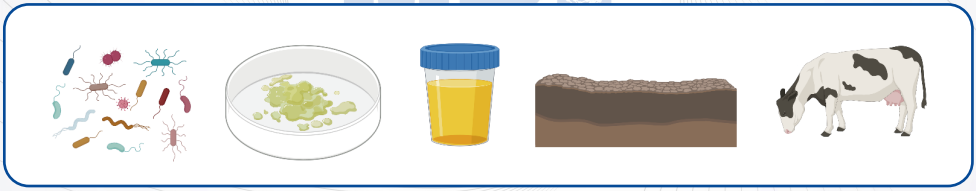
Flow-Chart



Summary of sample preparation in Lysing Matrix E

Sample Type	Optimal amount of sample usage	Pretreatment
Bacteria	50-100 mg (wet weight) or up to 10^{10} bacterial and 10^{8} yeast cells	Resuspend cell pellet in 800 µL of Buffer MB1 and transfer to Lysing Matrix E.
Sputum	<300 µL	Sputum added to Lysing Matrix E for lysis; for large volume sputum pretreatment, refer to manual.
Bronchoalveolar	1-10 mL	After centrifugation for 15 min @ 3,000 *g* (at 4 °C), recover the precipitate for lysis step.
Urine	1-20 mL	After centrifugation for 15 min @ 14,000 *g* , recover the precipitate for lysis step.
Milk	1-10 mL	Same as urine
Vinasse	200 mg	Add 800 µL Buffer MB1 directly for lysis.
Soil	100-500 mg	Same as vinasse



Pre-treatment of special samples

1. For difficult samples such as spores, increase lysis speed to 6-7 m/s; for easy samples, the lysis time can be reduced to improve efficiency.
2. Using the Buffer MB1, this kit can be used to extract DNA from oral/ nasal swabs directly and swabbing solution.
3. Refer to manual for specific sample processing methods.

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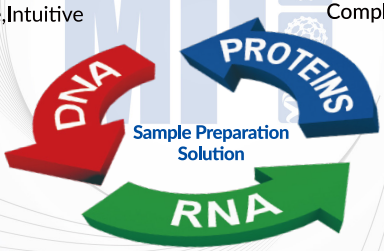

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