SPINeasy® DNA Kit for Microbiome

Cat. No.: 116553050 (50 preps) & 116553000 (5 preps)

Quick-Start Protocol

Revision 1.0 (June 2023)

Notes before starting:

- This kit allows 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.
- If there is any precipitate observed in Buffer MB1, heat at 37°C until the precipitate dissolves.
- Centrifugation speed stated on the manual is a guideline; use the maximum speed available if 15,000 x *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.

Lyse

1. Add 10^7-10^{10} cfu of bacterial sample into the Lysing Matrix E tube.
   Note: Refer to next page for recommended amounts and summary of sample preparation.

Remove inhibitor

2. Add **800 µL of Buffer MB1** and **25-40 µL of RNase A Solution**, invert the tubes several times to mix the lysing matrix and buffer. Homogenize in a **FastPrep®** instrument for 45 seconds twice at speed setting of 5 m/s with a 5 minutes interval. Centrifuge at ≥**15,000 x *g*** for 2 min.

3. Transfer all supernatant into a 2 mL centrifuge tube (self-provided). Add **300 µL of Buffer MB2**, invert and mix 5 times. Centrifuge at ≥**15,000 x *g*** for 2 min.

4. Transfer the supernatant (~750 µL) into a clean 2 mL centrifuge tube (self-provided). Add **750 µL of Buffer MB3**, invert and mix twice.

Bind

Microcentrifuge Method

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

Vacuum Manifold Method

5. Insert the Column MB into the vacuum manifold's luer connectors. Load **~750 µL** of 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 of Buffer MB4** into the center of the column and apply vacuum. Switch off the vacuum source.
8. Add **700 µL of Buffer MB5** by running the pipette tip along the wall of the column and apply vacuum.

Wash

9. Without addition of any liquid, centrifuge at **15,000 x *g*** for 2 min to dry the column.

Elute

10. Discard the collection tube and place the column MB into a new 1.5 mL Collection Tube (provided).
11. Add **45-50 µL** of **Buffer MB6** onto the center of the membrane column **SLOWLY**. Centrifuge at **15,000 x *g*** for 1 min. Reload the eluate or add **45-50 µL of fresh Buffer MB6** into the column. Spin for 1 min at maximum speed.
    Note: For maximum yield, the elution volume can be increased to 200 µL.

Lyse

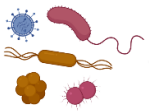
Remove inhibitor

Bind

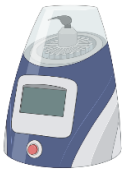
Wash

Elute

Microbial sample



Buffer MB1 (800 µL)
RNase A (25-40 µL)



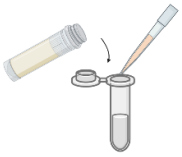
Or

45 s, 5 m/s; twice

10 min
2,500-3,000 rpm

15,000 x *g*, 2 min

Pour supernatant (~700 µL) into 2 mL tube

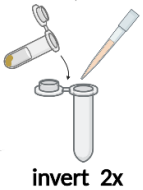


Buffer MB2 (300 µL)

invert 5x

15,000 x *g*, 2 min

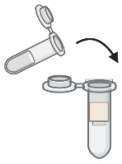
Pour supernatant into 2 mL tube



Buffer MB3 (750 µL)

invert 2x

Microcentrifuge Method



Pour 750 µL mixture into Column MB

15,000 x *g*, 1 min

DNA

Repeat

15,000 x *g*, 1 min

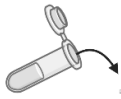
Buffer MB4 (500 µL)

15,000 x *g*, 1 min

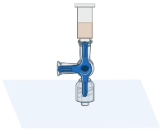
Buffer MB5 (700 µL)

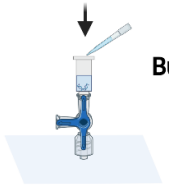
15,000 x *g*, 1 min

Vacuum manifold Method

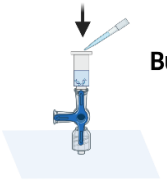


Pour mixture into Column MB

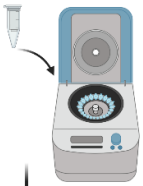




Buffer MB4 (500 µL)



Buffer MB5 (700 µL)

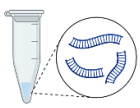


15,000 x *g*, 2 min

Repeat

Buffer MB6 (50 µL)

15,000 x *g*, 1 min



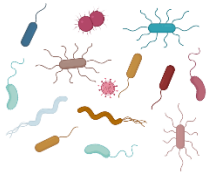
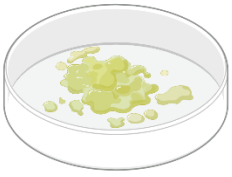
Highly purified genomic DNA

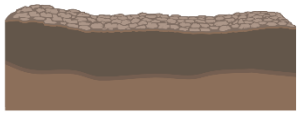
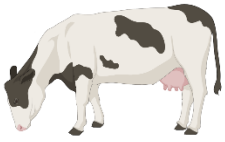
Scan for detailed instruction manual

www.mpbio.com

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 Lavage Fluid	1-10 mL	After centrifugation at 3,000 xg for 15 min (at 4°C), recover the precipitate for lysis step.
Urine	1-20 mL	After centrifugation at 14,000 rpm for 15 min, 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.