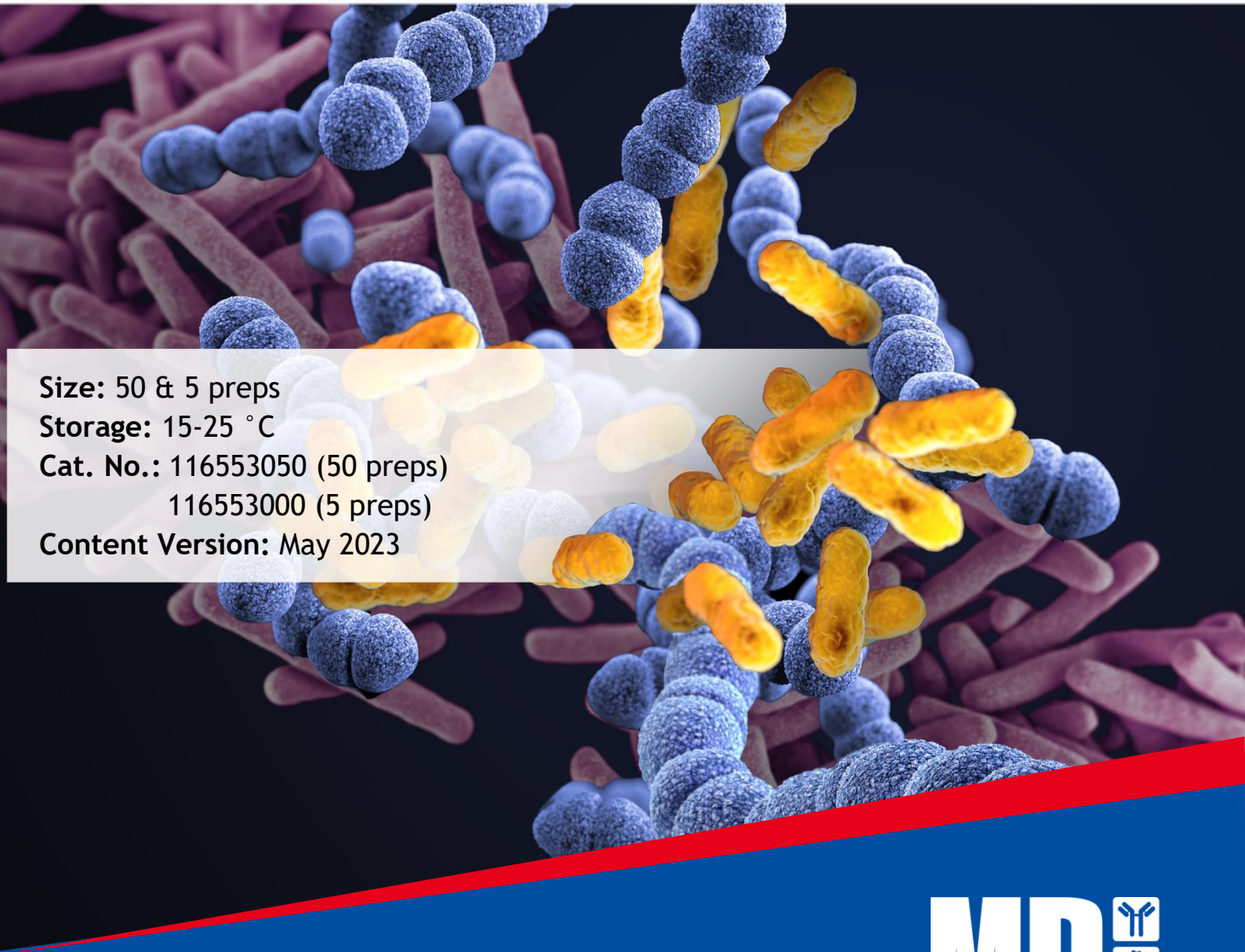
# Instruction Manual
Research Use Only

# SPINeasy® DNA Kit for Microbiome

For simple isolation of microbial genomic DNA from various samples



**Size:** 50 & 5 preps
**Storage:** 15-25 °C
**Cat. No.:** 116553050 (50 preps)
116553000 (5 preps)
**Content Version:** May 2023

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# Table of Contents

# 1. Introduction to SPINeasy® DNA Kit for Microbiome

SPINeasy® DNA Kit for Microbiome employs a novel method for isolating microbial genomic DNA from various samples. Due to its highly effective lysis capability and silica-membrane spin-column technology, the SPINeasy® DNA Kit for Microbiome can be used for bacterial, body fluids and environmental samples such as soil and stool. With the use of specially formulated Buffer MB1 and Lysing Matrix E in combination with FastPrep® Instruments from MP Biomedicals, an efficient lysis of various samples can be achieved within seconds. Provided in the kit, the Column MB and kit buffers are designed to deliver gDNA of high yield and purity, and compatible with downstream applications such as qPCR, restriction digestion, and sequencing.

Visit www.mpbio.com to explore additional products to support your research.

# 2. Kit Components and User Supplied Materials

## 2.1 SPINeasy® DNA Kit for Microbiome Component

| Components | 50 reactions (Cat. No. 116553050) | | 5 reactions (Cat. No. 116553000) | |
|---|---|---|---|---|
| | Package | Cat. No. | Package | Cat. No. |
| Equilibration Buffer | 12 mL | 116547059 | 1.2 mL | 116547009 |
| Lysing Matrix E | 50 ea | 116914050 | 5 ea | 116914005 |
| RNase A Solution | 2 mL | 116553058 | 200 µL | 116553008 |
| Buffer MB1 | 45 mL | 116553053 | 4.5 mL | 116553003 |
| Buffer MB2 | 17 mL | 116553054 | 1.7 mL | 116553004 |
| Buffer MB3 | 21 mL × 2 | 116553055 | 4.2 mL | 116553005 |
| Buffer MB4 | 28 mL | 116553056 | 3 mL | 116553006 |
| Buffer MB5 | 20 mL × 2 | 116553057 | 4 mL | 116553007 |
| Buffer MB6 | 10 mL | 116553051 | 1 mL | 116553001 |
| Column MB | 50 ea | 116553052 | 5 ea | 116553002 |
| 1.5 mL Collection Tubes | 50 ea | 116530060 | 5 ea | 116530010 |
| Quick-start Protocol | 1 ea | - | Available www.mpbio.com | - |
| Instruction Manual | Available www.mpbio.com | - | Available www.mpbio.com | - |
| MSDS & CoA | Available www.mpbio.com | - | Available www.mpbio.com | - |

## 2.2 User Supplied Materials

- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500)
- If FastPrep® Instrument is unavailable, a vortex (with an adapter for 1.5 mL / 2 mL tube) is recommended, especially when multiple samples are to be processed simultaneously.
- Microcentrifuge capable to work at the speed of ≥ 15,000 x *g*.
- 2 mL microcentrifuge tubes
- Vacuum Pump and Manifold (if vacuum method is to be used)

## 3. Storage and Kit Stability

The recommended retest date of all SPINeasy® DNA Kit for Microbiome components is 12 months from the date of manufacture when stored at room temperature (15 - 25 °C).

## 4. Important Consideration Before Use

Please check as appropriate:

- ❑ Prepare two pieces of 2 mL microcentrifuge tubes per prep for collection of flow-through.
- ❑ Lysis can be performed by vortexing the sample in Lysing Matrix E vial at 2,500 - 3,000 rpm if a FastPrep® Instrument is unavailable.
- ❑ Centrifugation speed stated in the manual is a guideline; use the maximum speed available if 15,000 x *g* is not feasible.
- ❑ Check Buffer MB1 for precipitation. If precipitation occurs, warm the buffer at 37°C until the precipitate dissolves.

## 5. Safety Precautions

Buffer MB3/ Buffer MB4/ Buffer MB5 contains a component 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 that is available at www.mpbio.com for additional details.

# 6. Protocol

**Things to do before starting:**

- If **Buffer MB1** has precipitate, warm it at **37°C** until the precipitate dissolves.
- Centrifugation speed stated in the manual is 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 - 20 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.

## 6.1 Protocol for Experienced User

**1. Column MB preparation**

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 the collection tube.

**2. Homogenization**

- Add sample into the **Lysing Matrix E** tube. (Refer to **6.2 Protocol Attachment** for suggestions on handling of various samples)
- Add **800 µL Buffer MB1** and **25-40 µL RNase A Solution** into the Lysing Matrix E tube containing the sample. Invert the tube several times to mix. Homogenize with a **FastPrep®** instrument twice, at speed setting of **5 m/s** for **45 sec** each, with a **5 min** interval. Centrifuge for **2 min @ 15,000 *g***.

***Note:*** *For samples with rich RNA content such as yeast, more RNA enzymes can be added for complete RNA removal. For bacteria that are easy to lyse, it can be homogenized for **45 sec once @ 5 m/s**. For biodiversity experiment, lysis at **5 m/s** for **45 sec twice** is recommended.*

**3. Purity**

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

**4. Binding**

- Transfer the supernatant (750 µL) to a clean 2 mL microcentrifuge tube (self-provided).

  Add 750 µL Buffer MB3 (ratio of 1:1), invert and mix twice.

### Microcentrifuge

- Transfer **750 µL of mixture** to Column MB.
- Centrifuge for **1 min @ ≥15,000 g** and discard the flow-through.
- Repeat the above steps until all the lysate has passed through the Column MB.

### Vacuum manifold

- Insert Column MB into the vacuum manifold's luer connector (may need Column adapter). Load **750 µL of mixture** into Column MB and apply vacuum.
- Repeat until all the lysate has been loaded. Switch off the vacuum source

**5. Washing**

- Add 500 µL Buffer MB4 onto the center of the column. Centrifuge for 1 min @ 15,000 g, discard the flow-through and place the column back into the same 2mL collection tube.
- Add 700 µL Buffer MB5 onto the center of the column. Centrifuge for 1 min @ 15,000 g, discard the flow-through and place the column back into the same 2mL collection tube.

- Add 500 µL Buffer MB4 to the center of the column and apply vacuum. Switch off the vacuum source.
- Add 700 µL Buffer MB5 by running the pipette tip along the wall of the column and apply vacuum. Switch off the vacuum source.

**6. Column Drying**

- Transfer the column into the same 2mL Collection Tube.
- Centrifuge at 15,000 g for 2 min to dry the column.

**7. Column Drying**

Discard the collection tube and place the column MB into new 1.5 mL Collection Tubes (provided).

***Optional:*** *Open Column MB and let it air-dry at room temperature for a few minutes to further improve DNA purity.*

Add **45-50 µL Buffer MB6** onto the center of the column membrane SLOWLY. Centrifuge for **1 min @ 15,000 *g***. Reload the eluate or add **45-50 µL** of fresh **Buffer MB6** into the same column and centrifuge for **1 min @** maximum speed. High quality DNA is now ready for use in downstream experiments.

***Note:*** *For maximum yield, the elution volume can be increased to **200 µL**.*

## 6.2 Sample processing method

1. **Microbial culture samples**

Culture volume of as low as **200 µL** can be used. For extraction of **200 µL** sample, no centrifugation is needed. The sample can be added directly into the Lysing Matrix E and follow the procedure in the above protocol.

(A) Microbial liquid culture samples

- Centrifuge 1-2 mL of bacterial liquid culture for **2 min @ 15,000 *g***. Discard the supernatant.

- Resuspend cell pellet with **800 µL Buffer MB1**, and transfer to lysing matrix E.

- Continue to follow Step 2 of the above protocol.

(B) Microbial solid culture samples

- Transfer **50-100 mg** of bacterial solid culture into lysing matrix E.

- Continue to follow Step 2 of the above protocol.

2. **Body fluid samples**

Different body fluid samples, including bronchoalveolar lavage fluid, urine, saliva, sputum etc. have been tested successfully with SPINeasy® DNA Kit for Microbiome.

*Note: User may determine whether **2-8°C** centrifugation is required during the pretreatment according to the experimental requirements.*

(A) Cerebrospinal Fluid (CSF), Bronchoalveolar Lavage Fluid (BALF) and Pleural Fluid

- Collect samples according to your laboratory guidelines and experimental needs.

- Centrifuge the sample (**1-10 mL**) for **15-20 min @ 3,000 *g*** (at **2-8°C** ) to pellet the microorganisms.

- Discard the supernatant carefully, avoid disturbing the pellet. Loss of pellet will result in decreased yield.

- Resuspend the microbial pellet with **800 µL Buffer MB1**, and transfer to lysing matrix E.

- Continue to follow Step 2 of the above protocol.

(B) Milk, Urine, Blood, Plasma and Serum

- Centrifuge the sample (**1-20 mL**) for **10-15 min @ 14,000 rpm** to pellet the microorganisms.

- Discard the supernatant carefully, avoid disturbing the pellet. Loss of pellet will result in decreased yield.

- Resuspend the microbial pellet with **800 µL Buffer MB1**, and transfer to lysing matrix E.

- Continue to follow Step 2 of the above protocol.

*Note: **100-200 µL** of samples can be directly added to lysing matrix E for extraction as per the above protocol.*

(C) Sputum

- Collect samples according to your laboratory guidelines and experimental needs.

- Dilute sputum (**> 300 µL**) 4-5 times with 2M NaOH (e.g., 1 mL sputum + 3 mL 2M NaOH or 1 mL sputum + 4 mL 2M NaOH). Vortex (about 20-40 mL of samples) for **2-5 min** until the diluted sputum becomes homogenous without clumps.

- Incubate the diluted sputum for **4-6 hours** at **4°C** .

- Centrifuge the sample for **10-15 min @ 3,000 *g*** to pellet the microorganisms.

- Discard the supernatant carefully, avoid disturbing the pellet. Loss of pellet will result in decreased yield.

- Resuspend the microbial pellet with **800 µL** of **Buffer MB1**, and transfer to lysing matrix E.

- Continue to follow Step 2 of the above protocol.

*Note: **Direct extraction of sputum (<300 µL) is recommended to ensure the integrity of DNA**.*

(D) Saliva

- Collect samples according to your laboratory guidelines and experimental needs.

- **100-200 µL** of saliva can be directly added to lysing matrix E for extraction as per the above protocol.

**3. Environmental Samples**

(A) Solid environmental sample (soil, feces, and vinasse)

- Weigh **100-500 mg** of sample and add it into lysing matrix E.

- Continue to follow Step 2 of the above protocol.

(B) Water/Air samples

- Filter samples through a desired filter (not provided). The volume of sample used depends on user's experimental needs and turbidity of the sample.

- Cut the filter into small pieces and transfer into lysing matrix E.

- Continue to follow Step 2 of the above protocol.

**4. Swab**

(A) Direct process swab

- Cut the swab into the lysing matrix E tube.

- Continue to follow Step 2 of the above protocol.

***Note:*** *Direct process swab method is recommended.*

(B) Indirect process swab

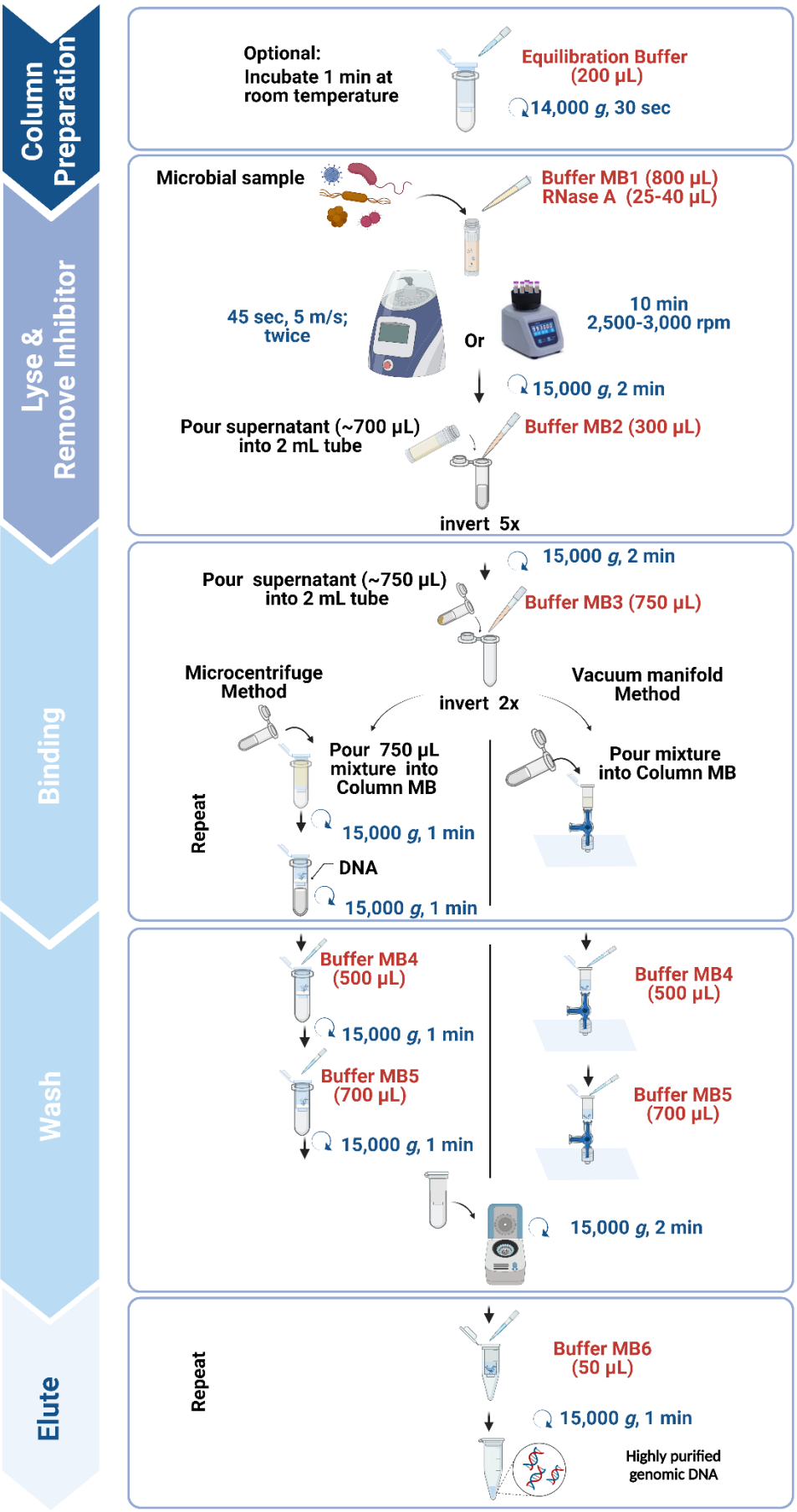
- Cut the swab into a 2 mL microcentrifuge tube.

- Add **750 µl Buffer MB1** into the tube and vortex for 1 min.

- Remove the swab and transfer solution into lysing matrix E.

- Continue to follow Step 2 of the above protocol.

## 7. Flow Chart


Column Preparation
Optional:
Incubate 1 min at room temperature
Equilibration Buffer (200 µL)
14,000 g, 30 sec
Lyse & Remove Inhibitor
Microbial sample
Buffer MB1 (800 µL)
RNase A (25-40 µL)
45 sec, 5 m/s; twice
Or
10 min
2,500-3,000 rpm
15,000 g, 2 min
Pour supernatant (~700 µL) into 2 mL tube
Buffer MB2 (300 µL)
invert 5x
Binding
15,000 g, 2 min
Pour supernatant (~750 µL) into 2 mL tube
Buffer MB3 (750 µL)
Microcentrifuge Method
Vacuum manifold Method
invert 2x
Repeat
Pour 750 µL mixture into Column MB
15,000 g, 1 min
DNA
15,000 g, 1 min
Pour mixture into Column MB
Wash
Buffer MB4 (500 µL)
15,000 g, 1 min
Buffer MB5 (700 µL)
15,000 g, 1 min
Buffer MB4 (500 µL)
Buffer MB5 (700 µL)
15,000 g, 2 min
Elute
Repeat
Buffer MB6 (50 µL)
15,000 g, 1 min
Highly purified genomic DNA

# 8. Data

SPINeasy® DNA Kit for Microbiome has been rigorously tested for its performance. The following are the results of gDNA extraction using SPINeasy® DNA Kit for Microbiome. DNA extracted is quantitated by fluorometric method and evaluated using agarose gel electrophoresis and qPCR.

**Microbial culture samples**

The bacterial DNA extracted with SPINeasy® DNA Kit for Microbiome has higher yield and better purity as compared to the competitor kits.

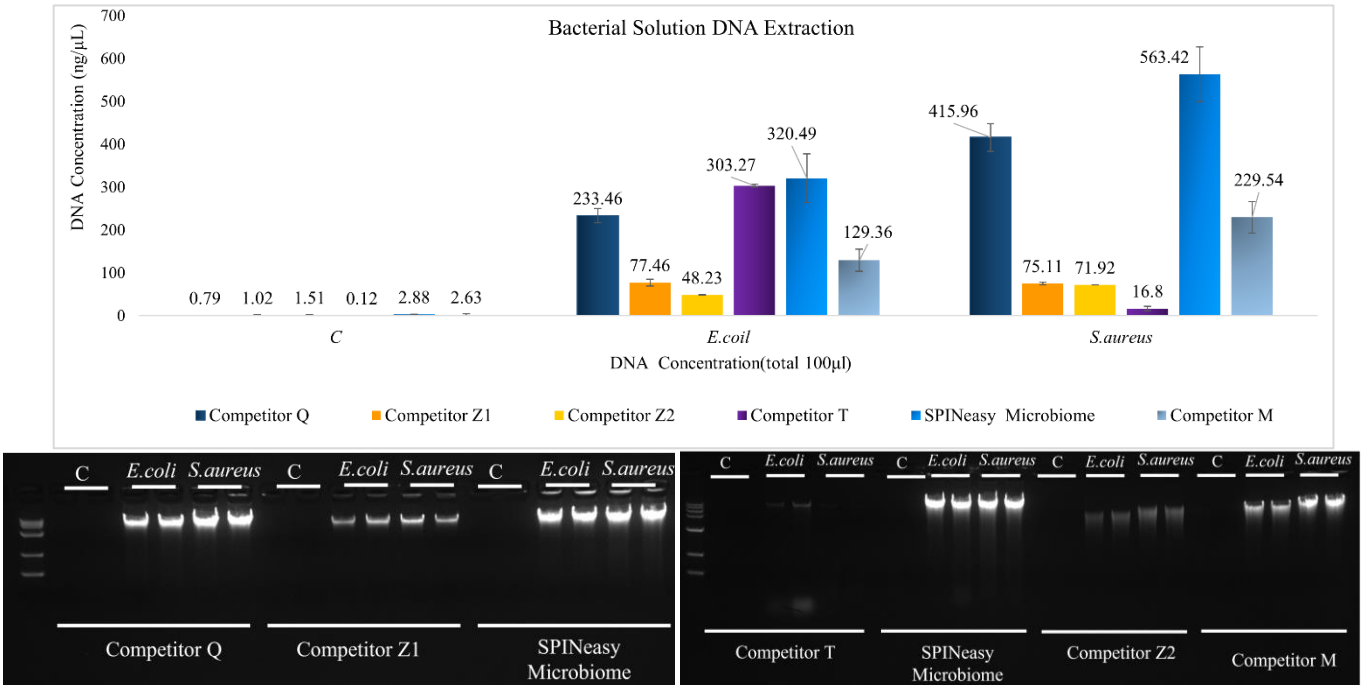



**Figure 1:** Quality and quantity of gDNA extracted from two different strains of bacteria (~$10^8$ cfu) using SPINeasy® DNA Kit for Microbiome and comparison with competitors. DNA marker: 1,5000 bp.

### Bronchoalveolar lavage fluid (BALF)

The DNA extracted with SPINeasy® DNA Kit for Microbiome has high yield and high molecular weight (indicating good DNA integrity).

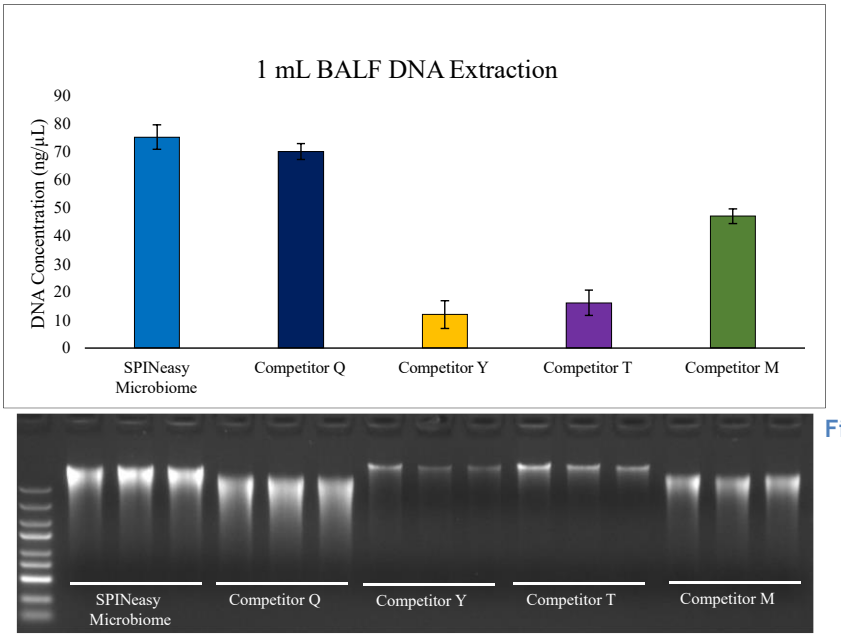
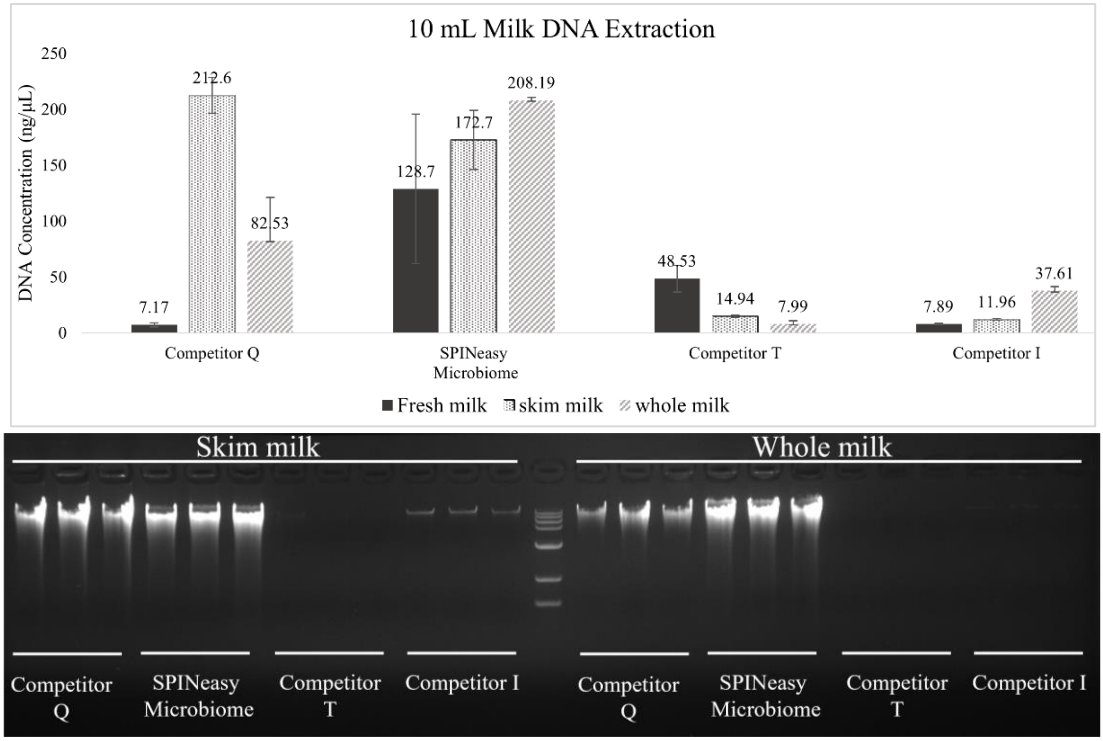



**Figure 2.** 1 mL BALF was used for DNA extraction. SPINeasy® DNA Kit for Microbiome produced the highest yield and least degradation of DNA as compared to the tested competitor kits. The results of the three replications were consistent. DNA marker: 1,5000 bp.

### Milk

Different types of milk contain different amount of protein and fat. SPINeasy® DNA Kit for Microbiome has an effective impurity removal system and is able to extract gDNA from the three types of milk tested. The extracted DNA showed high yield and better purity than the competitor kits.



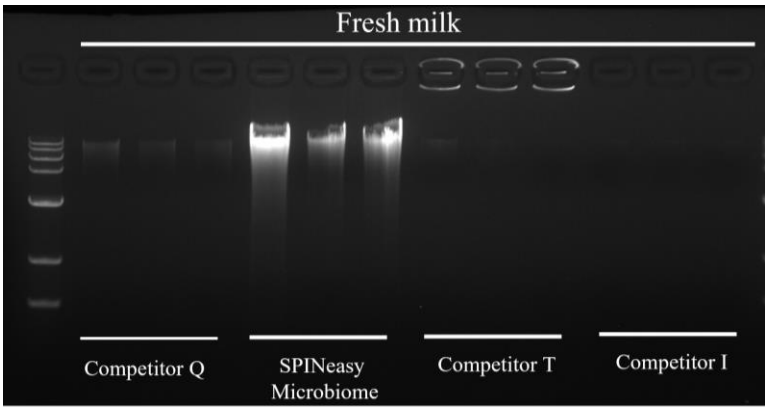
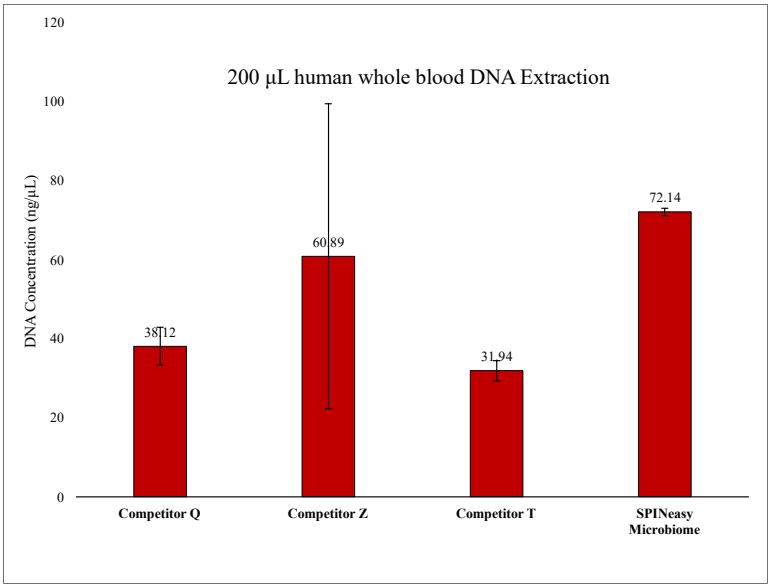
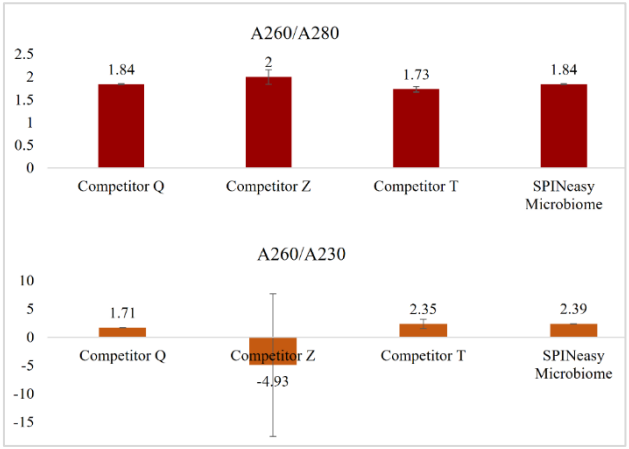



**Figure 3.** SPINeasy® DNA Kit for Microbiome gave high DNA yield and purity for the three types of milk. 10 mL milk spiked with 500 µL of bacterial solution were used. DNA marker: 1,5000 bp.

### Human whole blood

200 µL of human whole blood spiked with bacterial solution were directly added to **Lysing Matrix E** for DNA extraction. The DNA extracted with SPINeasy® DNA Kit for Microbiome showed high yield and purity.



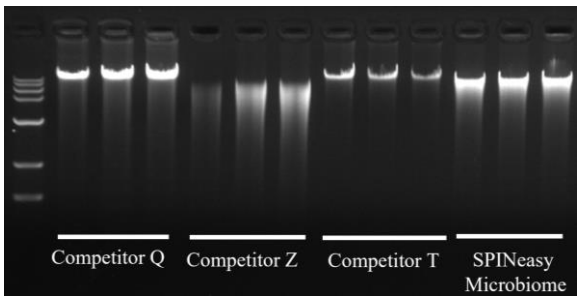




**Figure 4.** 200 µL Human whole blood (spiked with 10 µL Bacterial Solution) was used for DNA extraction. SPINeasy® DNA Kit for Microbiome produced the highest yield and least degradation of DNA as compared to the tested competitor kits. as compared to competitor kits. The results of the three replications were consistent. DNA marker: 1,5000 bp

### DNA qPCR

The DNA extracted with SPINeasy® DNA Kit for Microbiome could directly be used for downstream applications (such as qPCR), suggesting the effectiveness of the kit in removing the inhibitors.

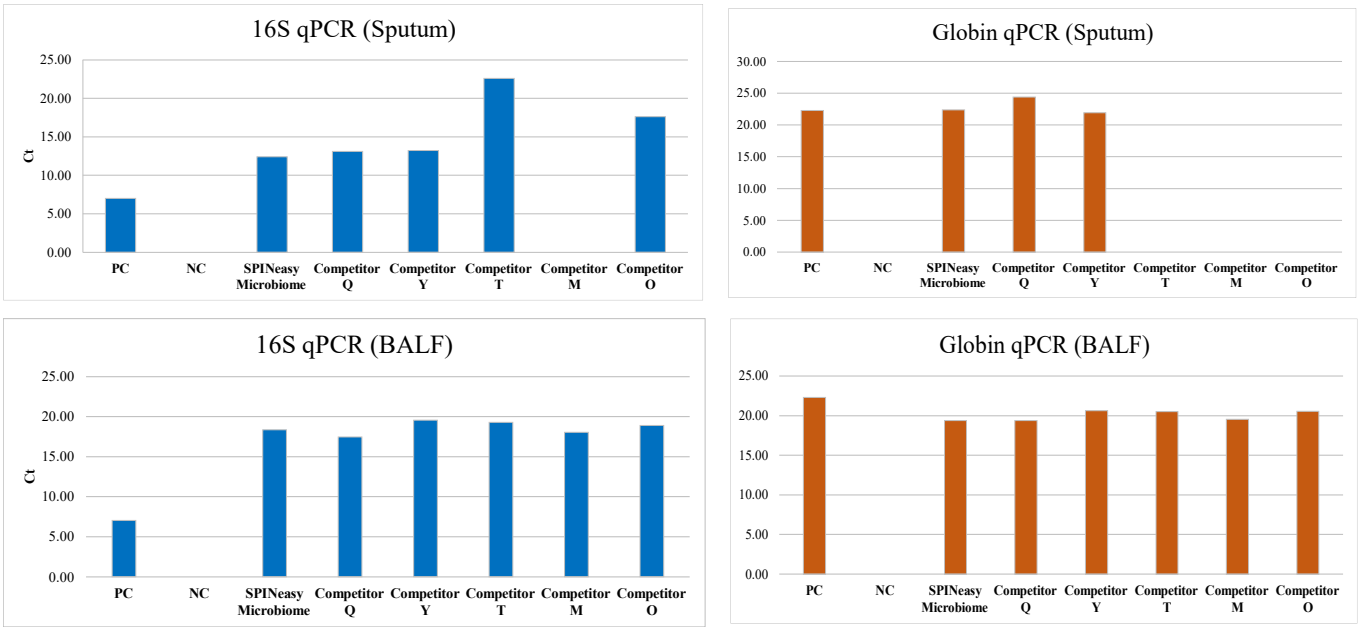



Figure 5. qPCR amplification of gDNA extracted from Sputum and BALF samples using SPINeasy® DNA Kit for Microbiome. DNA template amount: 20 ng. Primers: 16S rRNA (197 bp) and Globin (400 bp).

# 9. 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 |
|---|---|---|
| Low DNA Yield | Insufficient lysis | Difficult samples such as spores and some fungi may require more vigorous lysis.<br>Use different FastPrep® setting e.g., increase FastPrep®-24 5G lysis speed to 6-7 m/s from the recommended 5 m/s. Approximately 15% more genomic DNA can be expected.<br>*Note: More vigorous lysis may result in different degrees of DNA degradation depending on sample types. Partially degraded DNA may lead to suboptimal amplification of large DNA fragments but should not affect qPCR performance. Alternatively, vortexing method can be used if DNA of better integrity is preferred.* |
| | For maximum DNA recovery of samples with low DNA content | Process multiple samples using several Lysing Matrix tubes before pooling the eluates. |
| | Poor elution | (i) Ensure Buffer MB6 is added onto the center of the column membrane.<br>(ii) Incubate for 2-5 min after addition of Buffer MB6 to column before centrifugation.<br>(iii) Buffer MB6 may be pre-heated to 56℃ prior to use.<br>(iv) Elute with a larger volume of Buffer MB6 and elute twice (total 200 µL). |
| | Ethanol carry-over to eluate | Incubate column at 55℃ for 1-3 min to dry the membrane completely before elution. |
| | Suboptimal storage conditions affecting performance of column | Reinstate the performance of column by adding 500 µL of 1M NaOH to the column prior to use. Centrifuge at 15,000 x *g* for 1 min. Discard the eluted NaOH and use the column according to the protocol. |
| Background contamination | Consumables, equipment and/or workspace were contaminated due to poor laboratory practices. | Ensure proper user training and decontamination of necessary items before and after use. |
| | Contaminant not removed efficiently | Incubate the column at room temperature for 1 min after the addition of Buffer MB2 (Step 3 of protocol). |
| | Colored Eluate | Insufficient washing. Repeat Buffer MB5 washing step if a colored flow-through is |

| | | |
|---|---|---|
| | | observed. |
| Smeared DNA bands | Over lysis of sample | (i) To optimize the FastPrep® lysis speed and/or duration for different sample types.<br>(ii) Lysis using a vortex instead of a FastPrep® will generally result in better DNA integrity but compromised yields. |
| | Sample degradation | Sample quality is critical to the integrity of purified DNA. For best results, DNA should be extracted from fresh samples or freshly frozen samples. It is recommended to store frozen samples in aliquots and avoid repeated freeze-thawing. |
| Poor PCR Performance | High concentration of DNA | Dilute the sample. Large amount of DNA sample is inhibitory for PCR. Large amount of DNA molecule in the confined space of the reaction vessel is known to lead to false priming, exhaustion of the magnesium ions, primer(s), dNTP(s) and obstruct the passage of the large Taq polymerase molecules. If PCR using undiluted sample is required, check enzyme specification and manufacturer instruction or choose alternative PCR enzyme with strong strand displacement activity. If PCR can be done using diluted sample, the amount of DNA to be used is specific to each PCR enzyme and may need to be optimized by the user. However, genes in multiple copies in the genome such as ribosomal genes require much lesser DNA input.<br>SPINeasy® DNA Kit for Microbiome allows positive amplifications from various samples using as much as 200 ng or as little as 0.20 ng of DNA per 20 µL of PCR reaction. |
| | Suboptimal PCR condition | Verify PCR reagents and protocol with positive control; adjustment on reaction/cycle conditions or primer selection may be necessary following manufacturer recommendation. |

# 10. 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 in order 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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