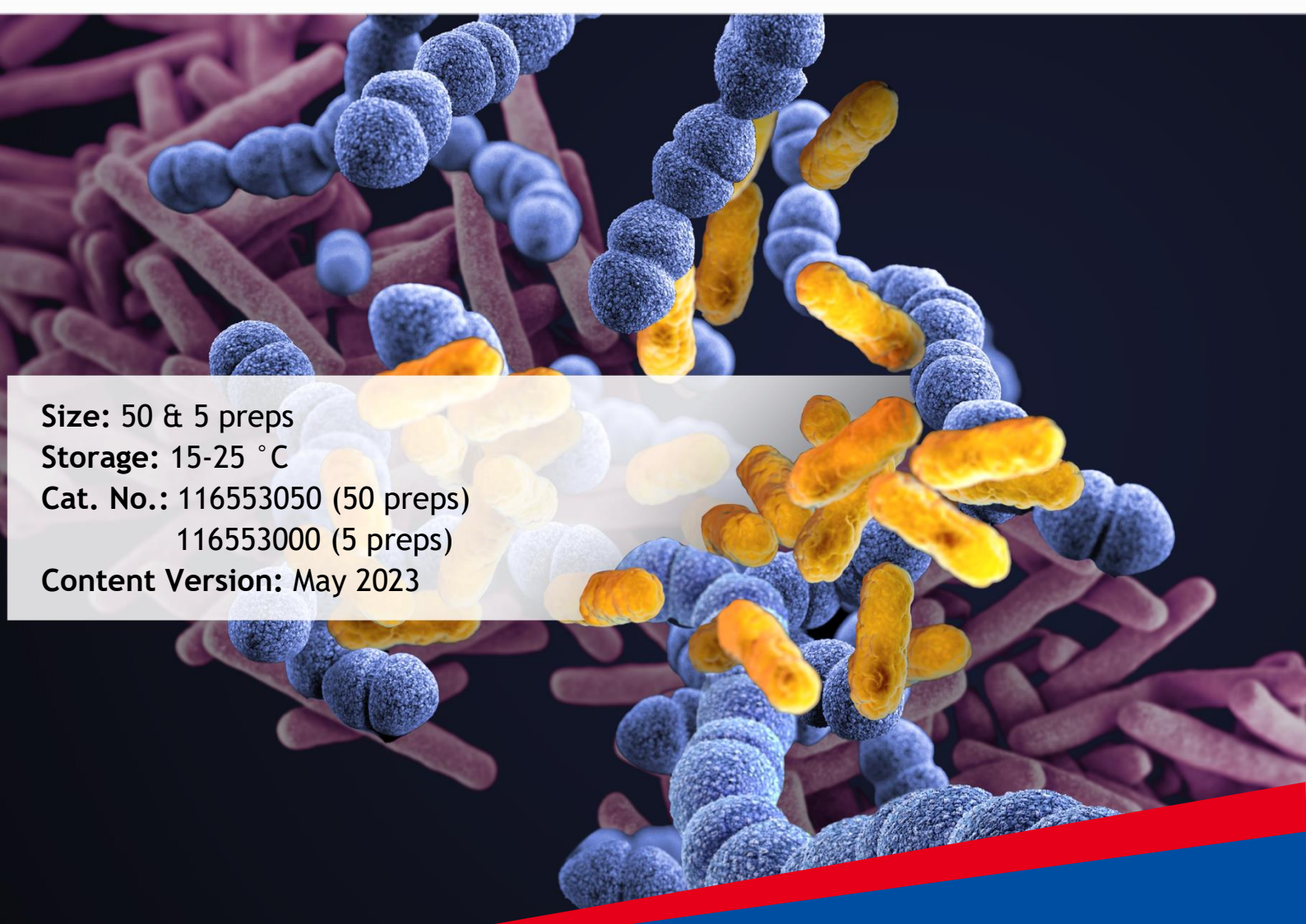


SPINeasy[®] DNA Kit for Microbiome

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

Table of Contents

1. Introduction to SPINeasy® DNA Kit for Microbiome	3
2. Kit Components and User Supplied Materials.....	4
3. Storage and Kit Stability.....	5
4. Important Consideration Before Use.....	5
5. Safety Precautions	5
6. Protocol.....	6
7. Flow Chart.....	10
8. Data	11
9. Troubleshooting	15
10. Product Use Limitation & Warranty.....	17

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.
Lysing Matrix E	50 ea	116914050	5 ea	116914005
RNase A Solution	2 mL	116546051	200 µL	116546001
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 $\geq 15,000 \times g$.
- 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 x 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. Lyse

- Add sample into the **Lysing Matrix E** tube. (Refer to **6.2 Protocol Attachment** for suggestions on handling of various samples)
- Add **800 µL of Buffer MB1** and **25-40 µL of 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 at **≥15,000 x g** for 2 min.

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 at 5 m/s. For biodiversity experiment, lysis at 5 m/s for 45 sec twice is recommended.

2. Purity

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

3. Binding

- Transfer the supernatant (750 µL) to a clean 2 mL microcentrifuge tube (self-provided). Add **750 µL of Buffer MB3** (ratio of 1:1), invert and mix twice.

Microcentrifuge

- Transfer **750 µL** of mixture to Column MB.
- Centrifuge at **≥15,000 x g** for 1 min and discard the flow-through. Repeat the above steps until all the mixture 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 mixture has been loaded. Switch off the vacuum source to avoid membrane over drying.

4. Wash

- Add **500 µL of Buffer MB4** onto 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.
 - Add **700 µL of Buffer MB5** onto 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.
- Add **500 µL of Buffer MB4** onto the center of the column and apply vacuum. Switch off the vacuum source.
 - Add **700 µL of Buffer MB5** by running the pipette tip along the wall of the column and apply vacuum. Switch off the vacuum source before removing the column from the luer connector.

5. Column drying

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

6. Elute

- Discard the collection tube and place the column MB into a new 1.5 mL Collection Tube (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 of Buffer MB6 onto the center of the column membrane SLOWLY. Centrifuge at 15,000 x g for 1 min. Reload the eluate or add 45-50 µL of fresh Buffer MB6 onto the same column and centrifuge for 1 min at 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 Protocol for Experienced User

1. Microbial culture samples

Culture volume of as low as 200 µL can be used. For extraction from 200 µL of 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 at 15,000 x g for 2 min. Discard the supernatant.
- Resuspend cell pellet with 800 µL of Buffer MB1, and transfer to lysing matrix E.
- Continue to follow Step 1 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 1 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) at 3,000 x g for 15-20 min (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 of Buffer MB1, and transfer to lysing matrix E.
- Continue to follow Step 1 of the above protocol.

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

- Centrifuge the sample (1-20 mL) at 14,000 rpm for 10-15 minutes 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 1 of the above protocol.

Note: 100-200 µL of samples can be directly added into 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 in 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 at 3,000 x g for 10-15 min 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 1 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 into lysing matrix E for extraction as per the above protocol.

3. Environmental Samples

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

- Weigh 250-500 mg of sample and add it into lysing matrix E.
- Continue to follow Step 1 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 1 of the above protocol.

4. Swab

(A) Direct process swab

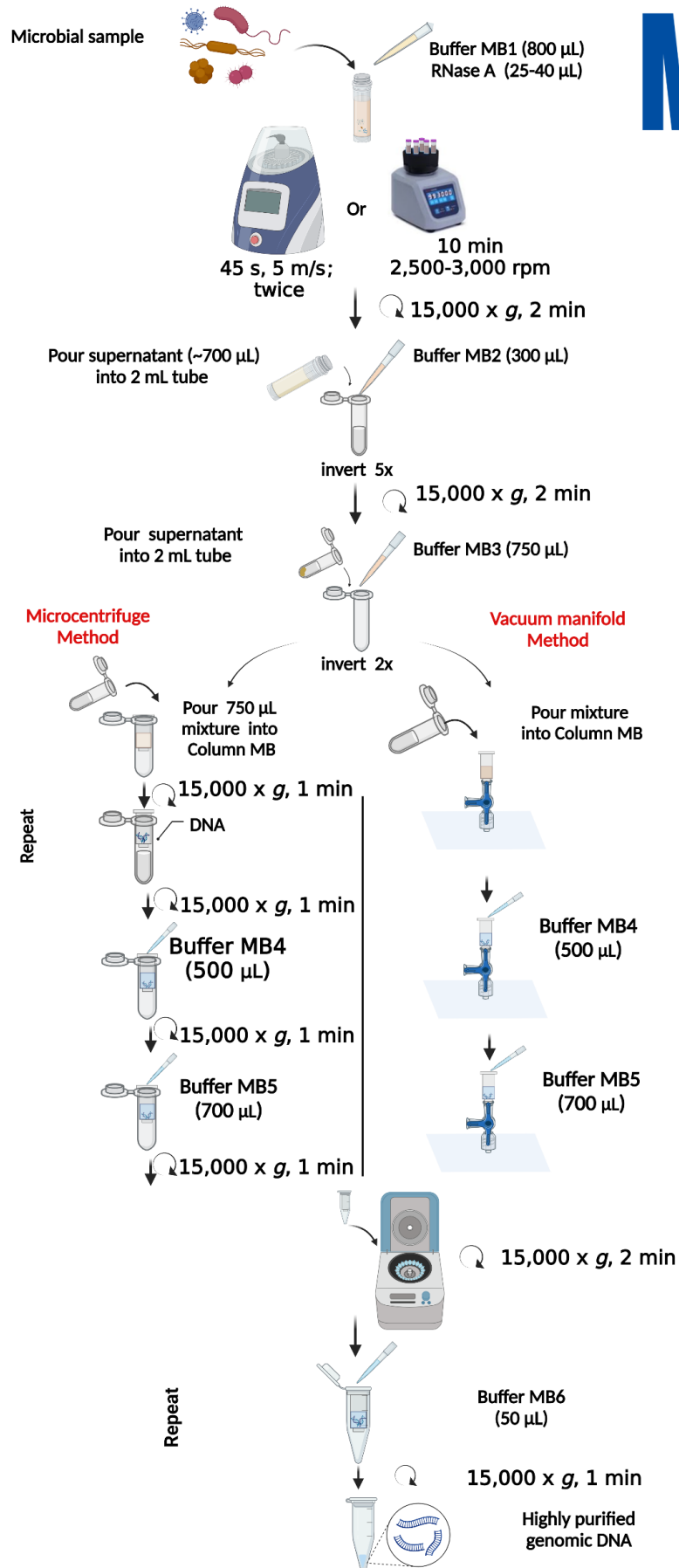
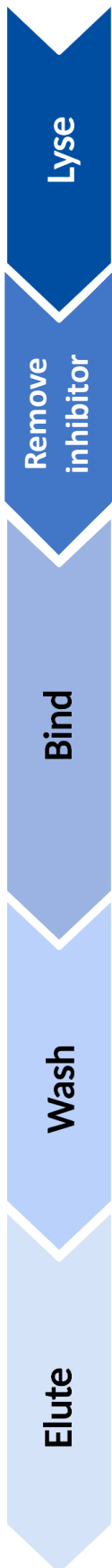
- Cut the swab into the lysing matrix E tube.
- Continue to follow Step 1 of the above protocol.

Note: Direct process swab method is recommended.

(B) Indirect process swab

- Cut the swab into a 2 mL microcentrifuge tube.
- Add 750 µL of Buffer MB1 into the tube and vortex for 1 min.
- Remove the swab and transfer solution into lysing matrix E.
- Continue to follow Step 1 of the above protocol.

7. Flow Chart



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. The extracted DNA was quantitated with 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.

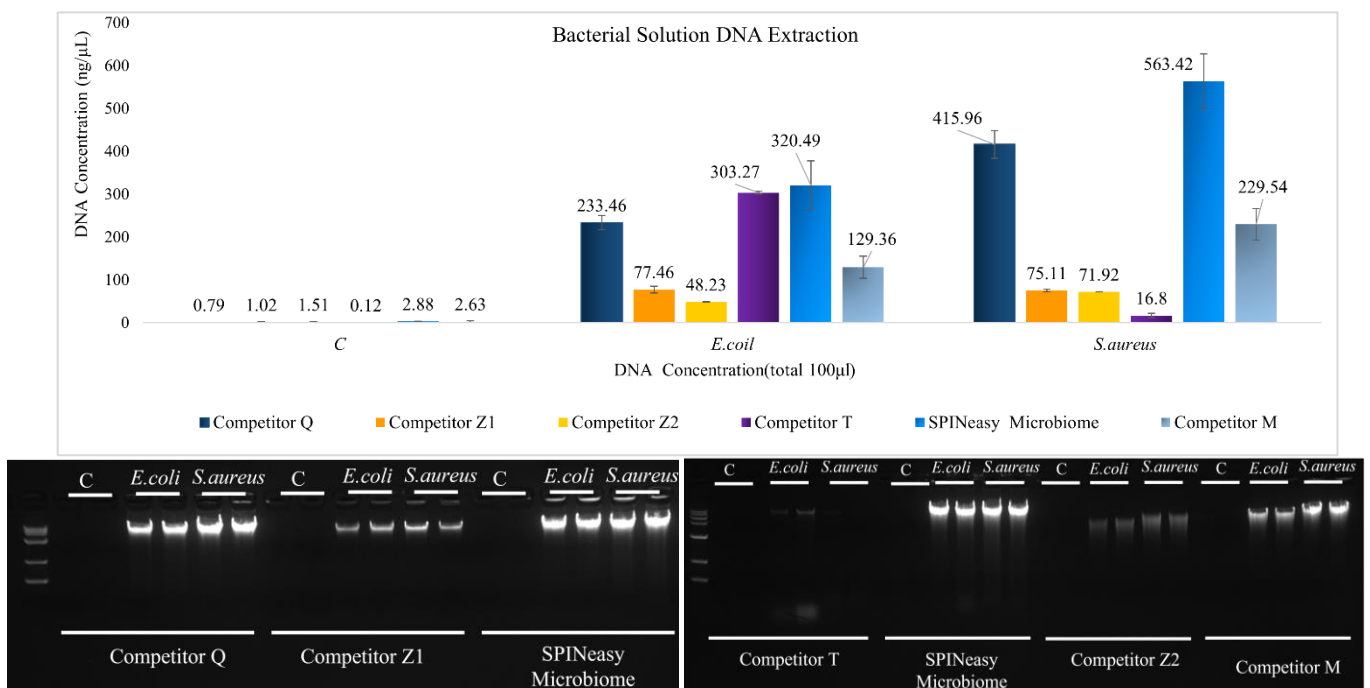


Figure 1: The SPINeasy® DNA Kit for Microbiome produced higher yield of DNA. Comparison of extraction of gDNA from two different strains of bacteria ($\sim 10^8$ cfu) using SPINeasy® DNA Kit for Microbiome and competitors kits. DNA marker: 1,500 bp.

Bronchoalveolar lavage fluid (BALF)

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

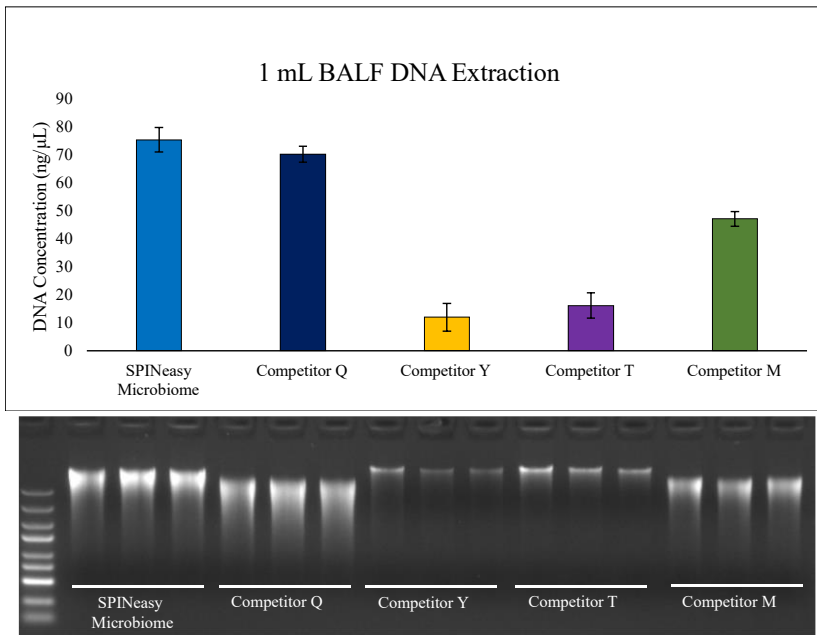
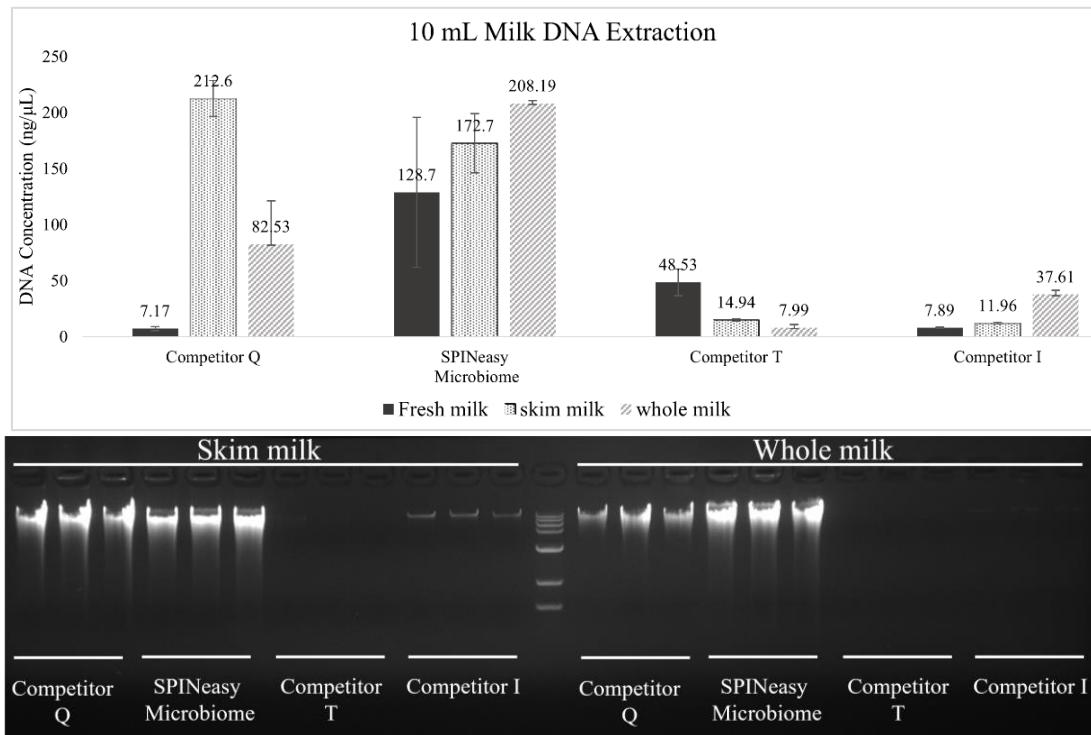


Figure 2. SPINeasy® DNA Kit for Microbiome produced the highest yield and least degradation of DNA as compared to the competitor kits. The results of the three replicates were consistent.

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.



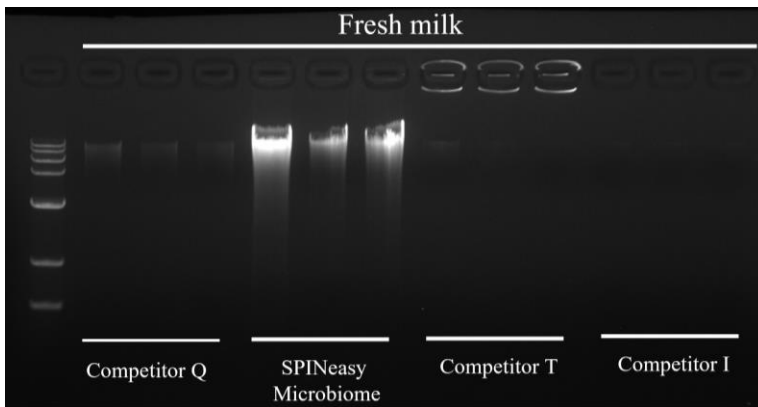


Figure 3. SPINeasy® DNA Kit for Microbiome gave high DNA yield and purity for the three types of milk. 10 mL of milk spiked with 500 µL of bacterial solution were used.

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.

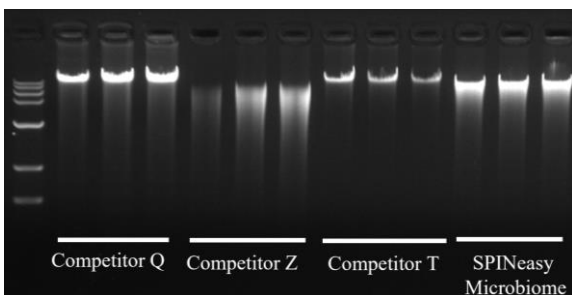
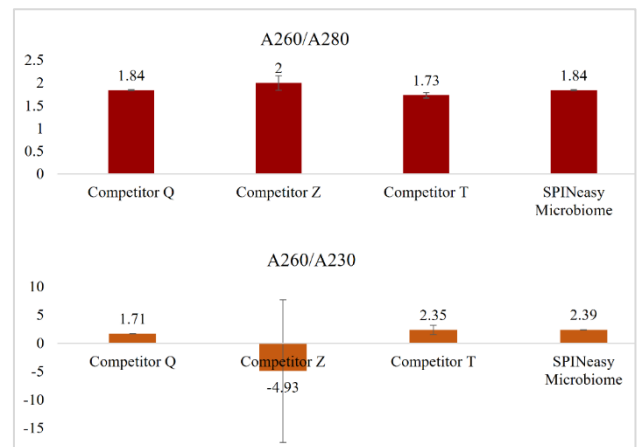
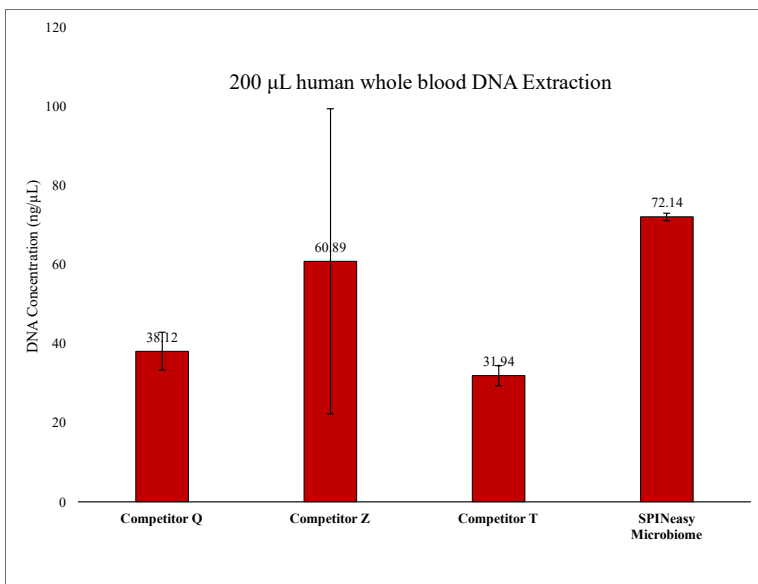


Figure 4. 200 µL of human whole blood (spiked with 10 µL of Bacterial Solution) was used for DNA extraction. SPINeasy® DNA Kit for Microbiome produced the highest yield and least degradation of DNA as compared to the competitor kits. The results of the three replicates were consistent.

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.

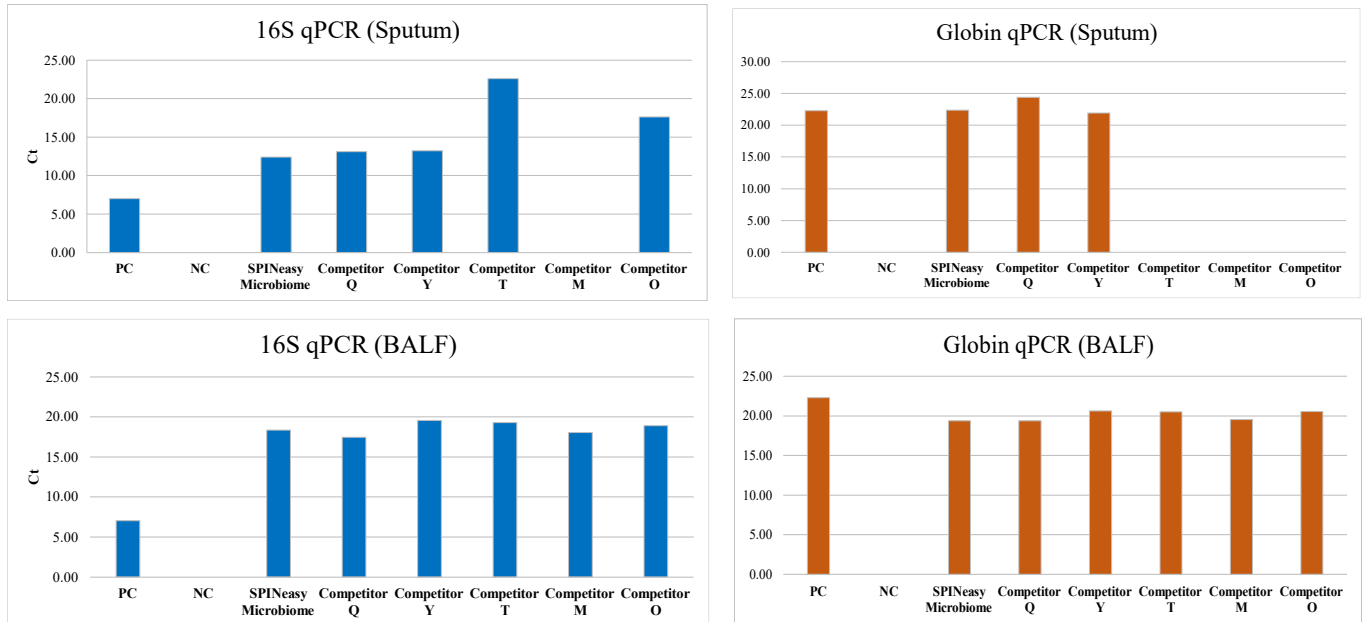


Figure 5. qPCR amplification of gDNA extracted from Sputum and BALF samples using SPINeasy® DNA Kit for Microbiome. DNA template amount: 20 ng. Primers: 16S rRNA (197 bp) and Globin (400 bp).

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. 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. <i>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.</i>
	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. (ii) Incubate for 2-5 min after addition of Buffer MB6 to column before centrifugation. (iii) Buffer MB6 may be pre-heated to 56°C prior to use. (iv) Elute with a larger volume of Buffer MB6 and elute twice (total 200 µL).
	Ethanol carry-over to eluate	Incubate column at 55°C 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.
Smear DNA bands	Over lysis of sample	(i) To optimize the FastPrep® lysis speed and/or duration for different sample types. (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. 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.

Buyer's exclusive remedy and the sole liability of MP Biomedicals hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification. By acceptance of the product, Buyer indemnifies and holds MP Biomedicals harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees, or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

Australia

Tel: +61 2.8824.2100
Tel: +61 1800.249.998
Email: custserv.au@mpbio.com

Austria & Germany

Tel: 0800.426.67.337
Tel: 00800.7777.9999
Email: custserv.de@mpbio.com

Belgium

Tel: 00800.7777.9999
Email: custserv.be@mpbio.com

Canada

Tel: +1 800.854.0530
Email: custserv.ca@mpbio.com

China

Tel: +86 400.150.0680
Email: custserv.cn@mpbio.com

Europe

Tel: +33 3.88.67.54.25
Tel: +33 00800.7777.9999
Email: custserv.eur@mpbio.com

France

Tel: +33 3.88.67.54.25
Email: custserv.fr@mpbio.com

India

Tel: +91 22.27636921/22/25
Email: custserv.in@mpbio.com

Italy

Tel: 00800.7777.9999
Email: custserv.it@mpbio.com

Japan

Tel: +81 3.6667.0730
Email: custserv.jp@mpbio.com

Latin America

Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.la@mpbio.com

New Zealand

Tel: +64 9.912.2460
Email: custserv.nz@mpbio.com

North America

Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.na@mpbio.com

Poland

Tel: 00800.7777.9999
Email: custserv.po@mpbio.com

Russia

Tel: +7 495 604.13.44
Email: custserv.rs@mpbio.com

Serbia

Tel: +381 11.242.1972
Email: custserv.se@mpbio.com

Singapore/ APAC

Tel: +65 6775.0008
Tel: +65 6394.7675
Email: custserv.ap@mpbio.com

South Korea

Tel: +82 2.425.5991
Email: custserv.kr@mpbio.com

Switzerland

Tel: 00800.7777.9999
Email: custserv.ch@mpbio.com

The Netherlands

Tel: 00800.7777.9999
Email: custserv.nl@mpbio.com

United Kingdom

Tel: 0800.282.474
Email: custserv.uk@mpbio.com

www.mpbio.com

