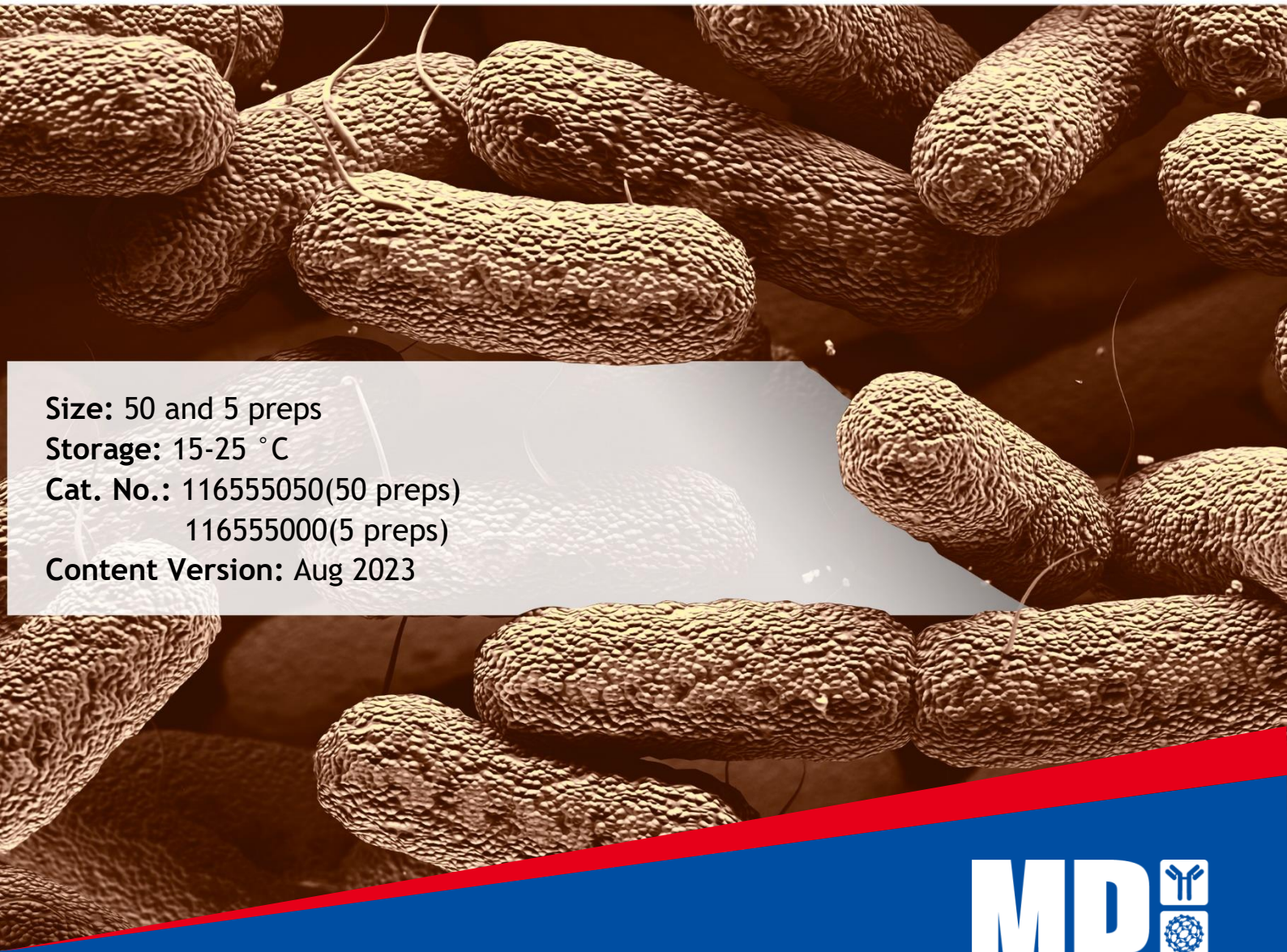
Research Use Only

Instruction Manual

SPINeasy® DNA/RNA Kit for Feces

For simultaneous extraction of microbial DNA and RNA from the same fecal sample



Size: 50 and 5 preps
Storage: 15-25 °C
Cat. No.: 116555050(50 preps)
116555000(5 preps)
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1. Introduction to SPINeasy® DNA/RNA Kit for Feces

The composition of fecal samples is largely dependent on diet which includes fibers, undigested particles, bilirubin, complex polysaccharides, and lipids. Those compounds impair the sample homogenization, decrease both the quantity and quality of the extracted DNA. The SPINeasy® DNA/RNA Kit for Feces integrates our proprietary inhibitor removal expertise and our new technology for selective binding of DNA and RNA. This kit is especially suitable when the sample is limited or when both genomic and transcriptomic manipulations are needed. This thereby avoids any potential variation in microbial community composition from processing bias.

The SPINeasy® DNA/RNA Kit for Feces allows simultaneous isolation of pure microbiome genomic DNA and RNA from challenging feces types irrespective of their composition. Isolated samples showed no contaminants and were immediately ready for use in downstream applications, including qPCR and RT-qPCR without the need of further inhibitor removal step.

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

Kit Specifications at a Glance

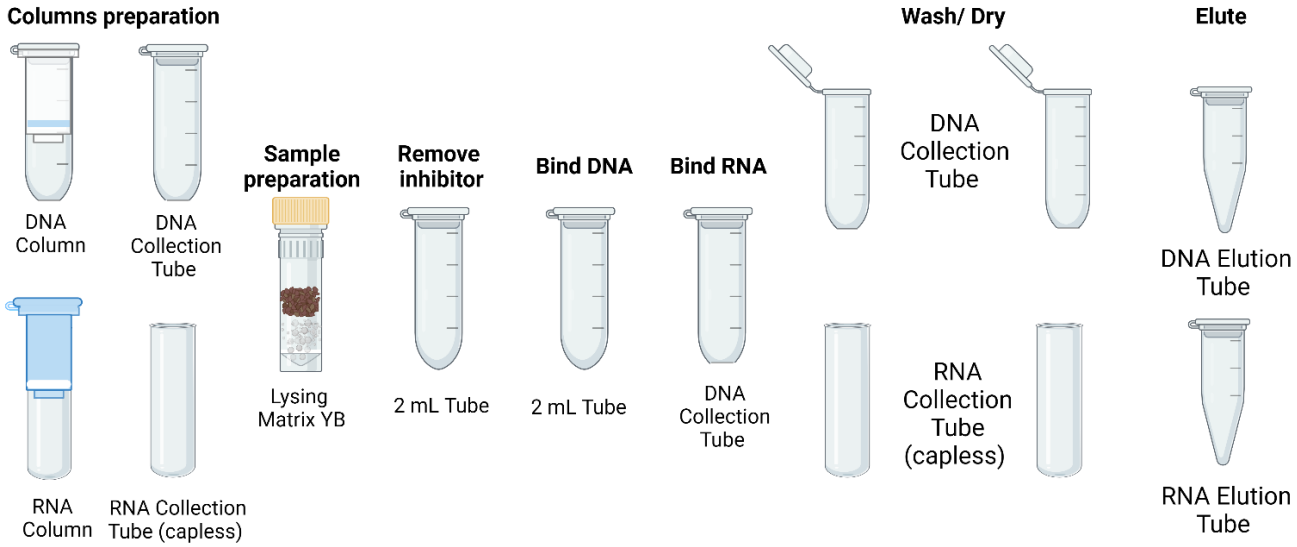
Technology	Silica membrane technology
Format	Mini Column
Sample	Feces (omnivore/ herbivore/ carnivore diets)
Sample amount	up to 250 mg.
Observed yield	up to 50 µg (DNA)/300 µg (RNA) (sample dependent)
Elution volume	30–100 µL
Preparation time	<45 min (6 preps)

2. Kit Components and User Supplied Materials

2.1 SPINeasy® DNA/RNA Kit for Feces Component

Product	50 reactions (Cat. No. 116555050)		5 reactions (Cat. No. 116555000)	
	Package	Cat. No.	Package	Cat. No.
Equilibration buffer	24 mL	116554063	2.5 mL	116554013
Lysing Matrix YB	50×2 mL	116547051	5×2 mL	116547001
Buffer FDR1	40 mL	116555051	4 mL	116555001
Buffer FDR2	15 mL	116555052	1.5 mL	116555002
Buffer FDR3	48 mL	116555053	4.5 mL	116555003
Buffer FDR4/DNA	42 mL	116555054	4 mL	116555004
Buffer FDR4/RNA	42 mL	116555055	4 mL	116555005
Buffer FDR5	72 mL	116555056	7.2 mL	116555006
RNase Free Water	5 mL*3	116554058	1.5 mL	116554008
DNA Column	50 EA	116554059	5 EA	116554009
RNA Column	50 EA	116554060	5 EA	116554010
2 mL Tube	100 EA	116555057	10 EA	116555007
DNA Collection Tubes	50 EA*5	116554064	25 EA	116554014
RNA Collection Tubes	50EA*3	116554061	15 EA	116554011
DNA Elution Tubes	50EA	116554065	5 EA	116554015
RNA Elution Tubes	50 EA	116554062	5 EA	116554012
Quick-Start Protocol	1 EA		1 EA	
MSDS & CoA	Available at www.mpbio.com	-	Available at www.mpbio.com	-

For faster processing, it is advised to pre-position the plasticwares and columns used during the extraction as depicted below.



2.2 User Supplied Materials

- Phenol-chloroform-isoamyl alcohol (25:24:1, pH 6.5-8.0) is required for this protocol.
- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500) or Vortex.
- Microcentrifuge capable of spinning at ≥ 15,000 x g.
- Single-channel pipettors (2 µL–1000 µL) and RNAse-free certified filter tips.
- Ethanol (96-100%). Do not use denatured alcohol.
- (Optional) a commercial vacuum manifold with luer connectors connected to a vacuum pump.

3. Storage and Kit Stability

The SPINeasy® DNA/RNA Kit for Feces is guaranteed until the expiry date stated on the kit when stored at room temperature (15-25˚C). For extended storage or storage in dry condition (humidity < 40 %), store the columns at **2-8˚C** to maintain their optimal performance.

Buffer **FDR2** needs to be stored at **2-8˚C** upon reception.

4. Important Consideration Before Use

- ❑ The SPINeasy® DNA/RNA Kit for Feces requires the use of a centrifuge capable of generating at least 15 000 *g* to obtain optimal results.
- ❑ An RNase-free environment is essential to obtain intact RNA samples.
  - Use proper microbiological, aseptic technique.
  - Hands and dust particles which carry bacteria and moulds are the most common sources of RNase contamination. Always wear disposable gloves when handling reagents and RNA samples and change them frequently.
  - Use the disposable plasticwares provided in the kit. The use of certified RNAse-free filter tips is highly recommended.
  - Keep tubes closed whenever possible.
  - Following extraction, keep purified RNA on ice for immediate use or store the samples at -80˚C.
- Gel electrophoresis: soak electrophoresis tanks, trays, and combs with 0.5% SDS solution, rinse with RNase-free water, then ethanol, and allow it to dry. Use electrophoresis buffer made with RNase-free water. Addition of *β-mercaptoethanol* such as MP Biomedicals Cat No. 02194834-CF (<0.5 µl) to the loading dye prior to mixing with the RNA sample as well as bleach solution (1%) to the melted agarose solution may help to preserve RNA integrity.

5. Safety Precaution

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. The Equilibration Buffer may cause skin burn and eye damage. Wear appropriate laboratory clothing including gloves and protective eyewear. Wear appropriate laboratory clothing including gloves and protective eyewear. Buffer FDR2 contains components that may cause irritation when in contact with human tissue. Buffers FDR3, FDR4, and FDR5 include chaotropic salts, which can form highly reactive compounds when combined with bleach. Buffers FDR3 and FDR5 are flammable.

6. Protocol

1. Column preparation

The DNA and RNA columns are pre-treated prior to usage to ensure their optimal performance. For this, transfer the DNA Column (capless) into a DNA Collection Tube **(provided).** Add **200 µL of Equilibration Buffer** to both **DNA** (capless) **and RNA (blue) Column membranes.**

Wait for at least **1 min** and centrifuge for **30 sec** @ maximum speed. Transfer the Column(s) into new DNA and RNA Collection Tubes (provided). The treated Column(s) can be stored at 2-8˚C for a few days, if required.

2. Sample preparation

Weigh up to **250 mg** of feces and add it to a **Lysing Matrix YB Tube.**

3. **Homogenization**

Mix the **Phenol: chloroform: isoamyl alcohol solution** (25:24:1, pH 6.5-8.0) thoroughly and add **100 µL** of the solution to the Lysing Matrix YB tube along with **650 µL** of **Buffer FDR1.**

Homogenize using **vortex @ 2500-2700rpm for 20 min** or **FastPrep® for 35 sec, 5 m/sec**. Centrifuge for **2 min @ maximum speed.**

Note: *The performance of the DNA output (yield, purity and DNA integrity) obtained using vortex is highly dependent on the model of vortex used. The condition stated above can be used as a starting point. The homogenization time and speed should be optimized by the user. Avoid using tape or handling the matrix tubes with your hands, which can result in reduced homogenization efficiency, inconsistent results, and reduced yields.*

4. Contaminant removal

Transfer the supernatant (~400-600 µL) into **2 mL Tube** (provided).

Optional: The Lysing Matrix YB Tube may be re-centrifuged after the transfer of the supernatant; any additional lysate can be recovered using a tip.

Add **200 µL of Buffer FDR2** to precipitate the contaminants, vortex for 1 sec and centrifuge for **2 min @ maximum speed.**

5.1 DNA binding

Transfer the supernatant into a **2 mL Tube** (provided). Add **1 volume** of **Buffer FDR3** and **vortex** for **1 sec**. For example, for 700 µL supernatant, add 700 µL of Buffer FDR3.

- The **DNA Column** (capless) shall be loaded in 2 steps as follows:

a) Pour **~750 µL** of the lysate into the **DNA Column** (capless). Centrifuge for **10 sec @ 15 000 *g***. The 2 mL Tube including the remaining lysate mixed with Buffer FDR3 can be centrifuged along to recover the mixtures found on the tubing lid and wall.

- Transfer the **DNA Column** (capless) to a new **DNA Collection Tube** (provided) and close the lid of the tube containing the first flow-through (it will be used for RNA purification).

b) Load the remaining lysate into the DNA Column (capless) and centrifuge for **10 sec @ 15 000 *g***.

- Transfer the **DNA Column** (capless) to a new **DNA Collection Tube** (provided) and combine the second flow-through with the first flow-through for RNA purification.

The RNA and DNA in the sample are present in the lysate flow-through and **DNA Column** (capless), respectively. The isolation of RNA or DNA can be performed separately if only one type of nucleic acid is required. To isolate RNA and DNA in two separate fractions, both subsequent steps can be performed simultaneously.

RNA purification

5.2 RNA binding

Add **250 µL of Ethanol** (not provided) to the lysate flow-through and mix immediately by pipetting up and down or vortex for 1 sec.
Pour **~825 µL** of the obtained supernatant into the **RNA Column** (blue), **centrifuge** for **10 sec @ 15,000 g** and discard the flow-through. Load the remaining mixture onto the column.

Optional: the DNA Collection Tubes containing the lysate-FDR3-Ethanol mix may be centrifuged along to recover the mixture found on the tubing lid and wall.

Repeat until all the lysate has been loaded through the **RNA Column** (blue).

Optional: for faster processing, vacuum manifold can be used. Insert the **RNA Column** (blue) into the vacuum manifold's luer connectors. Load **~825 µL** of the lysate into the RNA Column by decanting and **apply vacuum**. Repeat until all the lysate has been loaded.
Transfer the **RNA Column** (blue) to a new **RNA Collection Tube** (provided).

DNA purification

If both RNA and DNA are required, close the lid of the **DNA Column** (capless) to prevent it from drying out (until step 5 is performed). The column can also be stored at 2-8 °C for extended storage.

6. First Wash

! Two different wash buffers tailored for DNA and RNA extraction are used !. Add **600 µL** of **Buffer FDR4/DNA** to the center of the **DNA Column** (capless) and **600 µL** of **Buffer FDR4/RNA** to the center of the **RNA Column** (blue). Centrifuge for **10 sec @ 15 000 *g*,** discard the flow-through and place the Column(s) back into the same Collection Tube(s).

7. Second wash

Add **600 µL** of **Buffer FDR5** to the center of the Column(s), centrifuge for **30 sec @ 15 000 *g*.** Discard the flow-through and place the Column(s) back into the same Collection Tube(s).

8. Drying

Transfer the Column(s) into new DNA and/or RNA Collection Tube(s) (provided) and centrifuge for **1 min @ maximum speed.**

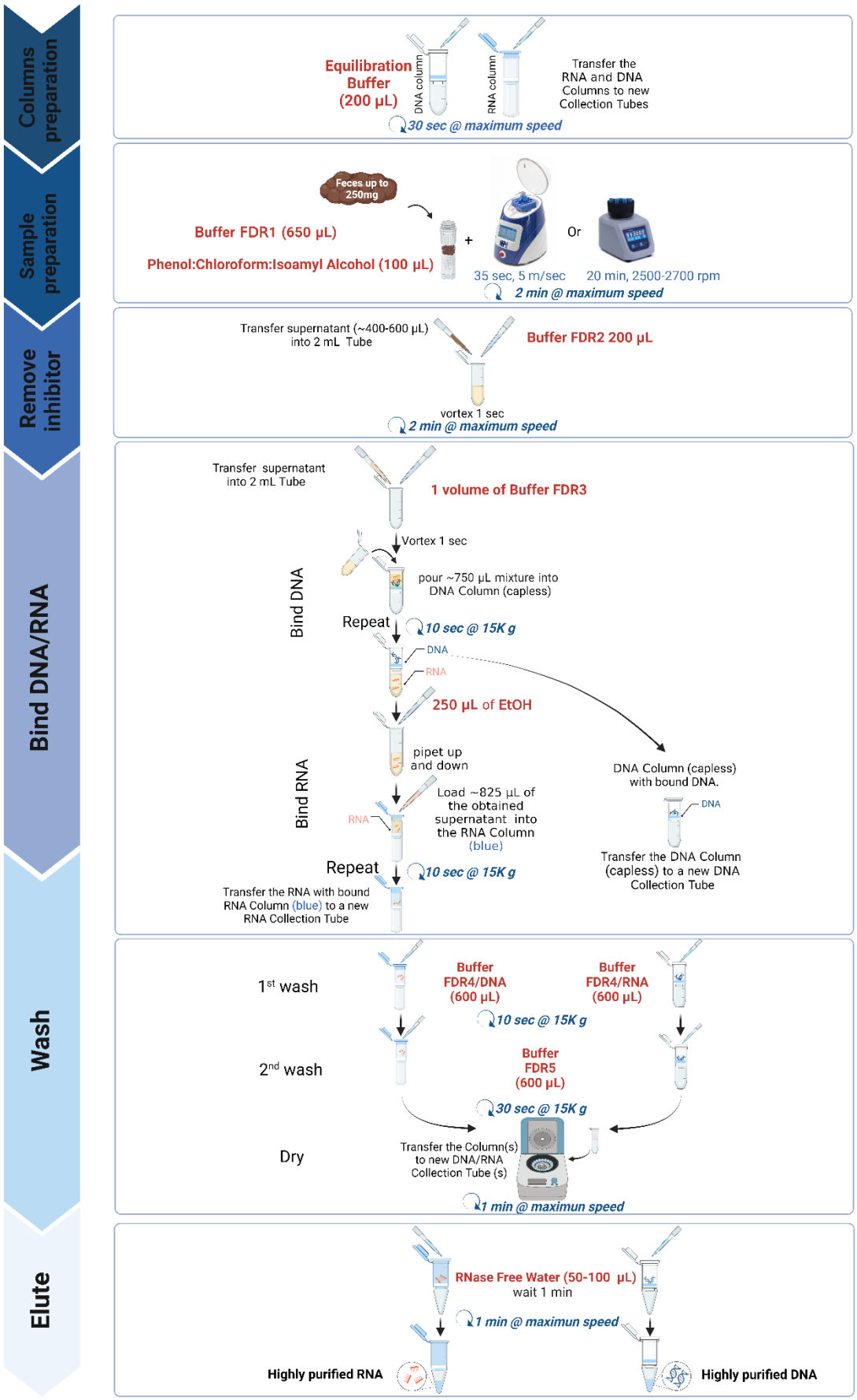
9. Elution

Transfer the Column(s) into new DNA and/or RNA Elution Tube(s) (provided). Add **100 µL of RNAse-free water** to the center of the membrane column, wait for **1 min** and centrifuge for **1 min @ maximum speed**. The RNA/DNA samples are now ready for downstream applications.

Note: Eluting with 100 µL of RNAse-free water will maximize the yield of nucleic acid. To obtain a more concentrated sample, a minimum of 50 µL of RNAse-free water can be used.

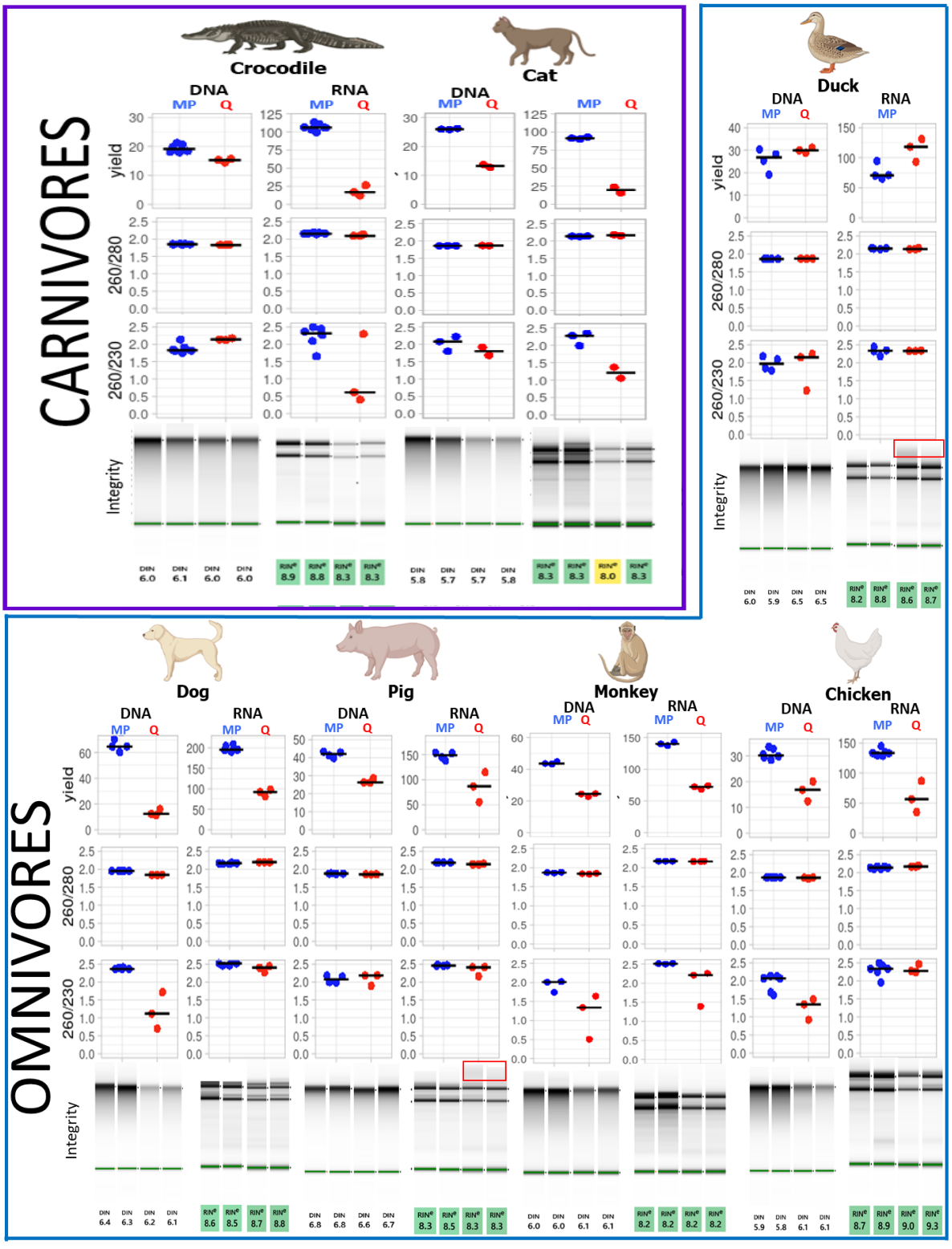
Note: The nucleic acid concentration of the sample is calculated by its UV absorbance at 260 nm where an absorbance of 1 (1 cm path length) is equivalent to 50 µL DNA/mL. Contamination with RNA, protein, salt, ethanol, humic acids or other non-nucleic acid contaminants contributes to the total absorption at 260 nm and therefore leads to an overestimation of the real DNA concentration. When measured using a UV spectroscopy, a ratio of A260/A280 between 1.80–1.90 and A260/A230 >1.8 indicates pure DNA. A260/A280 and A260/230 ratio above 2.0 indicate RNA contamination. Conversely, an A260/A280 ratio below 1.8 indicates protein contamination. Additionally, a low A260 / A230 ratio indicates the presence of humic acids as well as proteins, saccharides, ethanol, salt, and other contaminants which may inhibit subsequent enzymatic reactions.

7. Flow Chart


Columns preparation
Equilibration Buffer (200 µL)
DNA column
RNA column
Transfer the RNA and DNA Columns to new Collection Tubes
30 sec @ maximum speed
Sample preparation
Feces up to 250mg
Buffer FDR1 (650 µL)
Phenol:Chloroform:Isoamyl Alcohol (100 µL)
Or
35 sec, 5 m/sec
20 min, 2500-2700 rpm
2 min @ maximum speed
Remove inhibitor
Transfer supernatant (~400-600 µL) into 2 mL Tube
Buffer FDR2 200 µL
vortex 1 sec
2 min @ maximum speed
Bind DNA/RNA
Transfer supernatant into 2 mL Tube
1 volume of Buffer FDR3
Vortex 1 sec
Bind DNA
pour ~750 µL mixture into DNA Column (capless)
Repeat
10 sec @ 15K g
DNA
RNA
250 µL of EtOH
pipet up and down
Bind RNA
Load ~825 µL of the obtained supernatant into the RNA Column (blue)
Repeat
10 sec @ 15K g
Transfer the RNA with bound RNA Column (blue) to a new RNA Collection Tube
DNA Column (capless) with bound DNA.
Transfer the DNA Column (capless) to a new DNA Collection Tube
Wash
1st wash
Buffer FDR4/DNA (600 µL)
Buffer FDR4/RNA (600 µL)
10 sec @ 15K g
2nd wash
Buffer FDR5 (600 µL)
30 sec @ 15K g
Dry
Transfer the Column(s) to new DNA/RNA Collection Tube (s)
1 min @ maximun speed
Elute
RNase Free Water (50-100 µL)
wait 1 min
1 min @ maximun speed
Highly purified RNA
Highly purified DNA

8. Data

When used on various feces samples, the SPINeasy® DNA/RNA Kit for Feces delivered higher yield, optimal 260/280 and 260/230 ratio and similar or better integrity than the competitor Q kit, indicating a better extraction performance across a wide range of sample compositions (Figure 1).



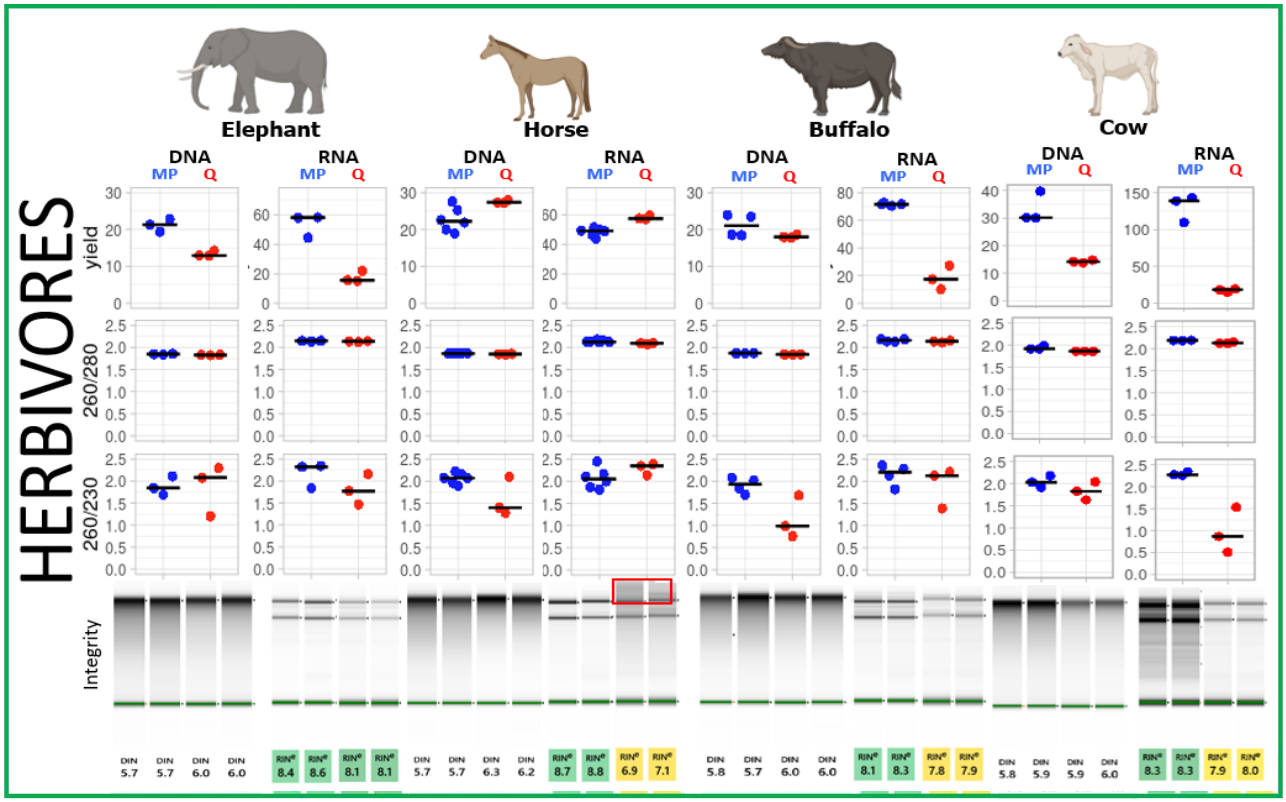


Figure 1. The SPINeasy® DNA/RNA Kit for Feces extracted DNA and RNA from various fecal samples with high yield and purity. Eleven fecal sample types were processed using SPINeasy® DNA/RNA Kit and competitor Q kit. Yield and purity ratios (260/280 and 260/230) were measured by fluorometric quantification. Each dot on the plot represents a single extraction. Representative virtual gels (obtained using Agilent 4150 TapeStation analyses) were presented together with the DNA integrity (DIN) and RNA integrity (RIN) values. The red rectangle indicates the contamination of RNA samples with some high molecular weight DNA.

Unlike competitor Q kit, no cross-DNA contamination was observed in RNA samples extracted with the SPINeasy® DNA/RNA Kit for Feces. As shown in Agilent virtual gel (Figure 1, red rectangle) or agarose gel profile (Figure 2), some RNA samples extracted with competitor Q kit contain DNA contamination; this highlights the outstanding performance

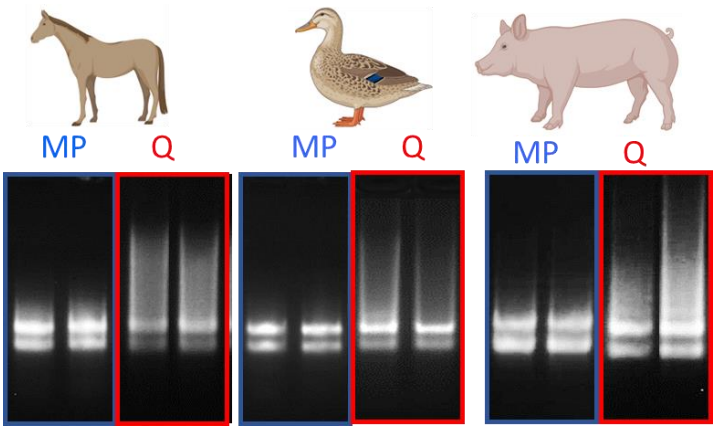


Figure 2. No DNA contamination in the RNA samples extracted with the SPINeasy® DNA/RNA Kit for Feces. RNA samples extracted from horse, duck and pig fecal samples were visualized on agarose gel. Unlike competitor Q, no smeared nucleic acid corresponding to DNA contamination could be observed in RNA samples extracted with MP Biomedicals' kit.

of the SPINeasy® DNA/RNA Kit for Feces as compared to other similar commercial kits in the market.

9. Troubleshooting

Problem	Possible Cause	Recommendation
Low nucleic acid yield / reduced integrity	Suboptimal homogenization	The time and/or speed used for FastPrep homogenization can be increased. ~15% more genomic DNA can be obtained when homogenizing with FastPrep® for 45 sec and 5m/sec instead of 35 sec. However, increasing DNA degradation may appear depending on the feces sample. Partially degraded DNA may lead to suboptimal amplification of large DNA fragment but should not affect qPCR performance. Alternatively, vortex can be used but may require further optimization.
	Nucleic acid is still bound to the Column	Wait for 10 min after addition of RNAse-free water before centrifuging. The RNAse-free water used for DNA elution may be pre heated to 70°C.
	The sample has been stored/frozen before extraction	Fresh sample is preferred to obtain optimal yield and integrity.
	RNase contamination	RNases are ubiquitous and stable laboratory contaminants and can be potentially introduced to a sample during experiment. Ensure that all the plasticwares used are RNase-Free. Follow the instruction stated on page 5 “Important Consideration Before Use”.
	Phenol:chloroform:isoamyl is oxidized	Phenol:chloroform:isoamyl alcohol is subject to oxidation reactions if improperly stored. Immediately close the Phenol:chloroform:isoamyl alcohol bottle after use and store it back at 4-8˚C. Avoid opening/closing of the bottle frequently. If the Phenol:chloroform:isoamyl alcohol turns pink or yellow, use a new stock (not provided) as coloured phenol: chloroform: isoamyl alcohol may result in compromised quality of extracted DNA and RNA.
Low A260/230 or A260/280 ratios	High level of contaminant in the sample	Reduce the amount of sample used and/or re-spin the supernatant obtained after inhibitor removal (step 4) for 1 min @ maximum speed and transfer it to a new 2 mL tube (not provided) before adding FDR3 to bind the DNA.
	Clogged Column	Reduce the amount of sample.
	Feces particles trapped on the Column’s membrane	Feces debris may remain in the supernatant despite the inhibitor removal step and be loaded onto the Column. Those debris can be removed by inverting the Column after drying and tapping the Column against the bench.

	Contamination of the DNA Column membrane	Ensure that all traces of wash buffer are removed from the rim of the Column prior to elution. A Kleenwipe ™ or a tip connected to the vacuum manifold (better) may be used. Alternatively, incubate the DNA Column with the lid open for 2 min at room temperature before adding the RNAse-free water
DNA contamination in RNA sample	Sample has high DNA content	For some feces with high DNA content, trace amount of DNA may still be found in the RNA lysate. Use less sample (containing less than 25 µg of genomic DNA), perform on DNA Column digestion using MP FastDNase I set. Following the wash using FR4/RNA, add 50 µL DNase I Solution into the center of the RNA Column (prepared by mixing 63 µL DNAse buffer and 2 µL FastDNase I enzyme). Incubate at room temperature for 1-2 min. Wash again using FR4/RNA buffer and proceed to step 7.
Poor PCR Performance	High concentration of nucleic acid	Dilute the sample. Large amount of nucleic acid sample is inhibitory for PCR. Large amount of DNA molecules 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/RNA Kit for Feces gave positive amplifications from various samples (from as much as >200 ng or as little as <0.20 ng of DNA per 20 µL reaction) using inhibitor sensitive PCR enzymes.
	Suboptimal PCR condition.	Verify PCR reagents and protocol with positive control; adjustment on reaction/cycle conditions or primer selection may be necessary following manufacturer's 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 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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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

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