Instruction Manual
Research Use Only

SPINeasy® DNA Pro Kit for Feces

For the isolation of genomic DNA from fecal samples

Size: 50 and 5 preps
Storage: 15-25 °C
Cat. No.: 116547050 (50) / 116547000 (5)
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1. Introduction to SPINeasy® DNA Pro Kit for Feces

Fecal samples display distinct processing challenges. 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 DNA extracted.

The SPINeasy® DNA Pro Kit for Feces was designed to tackle these challenges by providing an efficient way for rapidly isolating high-quality DNA from feces samples. Irrespective of the complexities, samples are optimally homogenized by bead beating with the new Lysing Matrix YB and lysis Buffer SF1. Subsequent treatment with Buffer SF2 effectively removes humic acid and other contaminants. The chemistry included in Buffer SF3 enables the specific binding of DNA without co-purification of RNA, eliminating the need for RNase A treatment. DNA obtained from fecal samples showed no inhibition in PCR and was immediately ready-to-be use for downstream applications, including long fragment PCR, qPCR, and next-generation sequencing (16S and whole genome) without the need for a further inhibitor removal step.

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Kit Specifications at A Glance

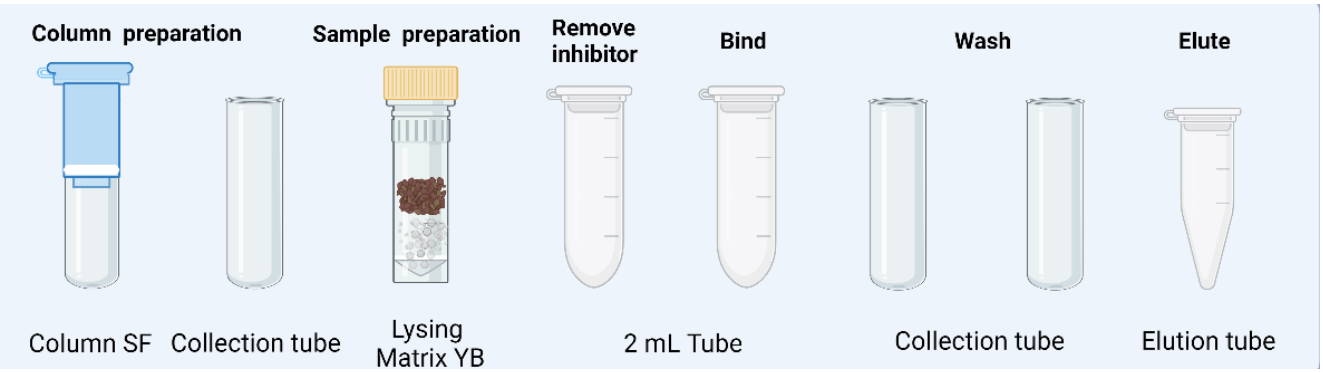
Technology	Silica membrane technology
Format	Mini spin column
Vacuum manifold	No
Sample	Feces (omnivore/ herbivore/ carnivore diets)
Sample amount	250 mg
Typical yield	up to 55 µg (sample dependent)
Elution volume	50–100 µL
Preparation time	<30 min (6 preps)

2. Kit Components and User Supplied Materials

2.1 SPINeasy® DNA Pro Kit for Feces Component

Product	50 reactions (Cat. No.116547050)		5 reactions (Cat. No.116547000)	
	Package	Cat. No.	Package	Cat. No.
Lysing Matrix YB	50 ea	116547051	5 ea	116547001
Equilibration buffer	12mL	116547059	1.2mL	116547009
Buffer SF1	54 mL	116547052	5.4 mL	116547002
Buffer SF2	12 mL	116547053	1.2 mL	116547003
Buffer SF3	48 mL	116547054	4.8 mL	116547004
Buffer SF4	40 mL	116547055	4 mL	116547005
Buffer SF5	40 mL	116547056	4 mL	116547006
Buffer SF6	7.5 mL	116547057	1 mL	116547007
Column SF	50 ea	116547058	5 ea	116547008
Collection tube	50 ea*3	116547060	15 ea	116547010
Elution tube	50 ea	116547062	5 ea	116547012
2 mL Tube	100 ea	116555057	10 ea	116555007
Quick-start Protocol	1 ea	-	1 ea	-
Instruction Manual	Available www.mpbio.com	-	Available www.mpbio.com	-
MSDS & CoA	Available www.mpbio.com	-	Available 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

- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500) or vortex mixer
- Microcentrifuge (speed ≥ 15,000 x g)
- Single-channel pipettors (2 µL–1000 µL)
- Nuclease-free tips

3. Storage and Kit Stability

All the SPINeasy® DNA Pro Kit for Feces components are guaranteed for 12 months upon receipt when stored at room temperature (15-25˚C). For extended storage, store the Column SF between 2-8˚C to maintain its performances. Column SF stored above 25 ˚C may result in reduced performance. The Buffer SF2 should be stored at 2-8˚C upon arrival.

4. Important Consideration Before Use

- ❑ Store the Buffer SF2 at 2-8˚C once received.
- ❑ A centrifuge able to generate at least 15,000 g is required to obtain optimal results. For step 2 (homogenization) and 3 (contaminant removal), perform centrifugation at the highest speed available.
- ❑ It is not recommended to use this kit with a vacuum manifold as this may decrease the overall purity.
- ❑ The SPINeasy® DNA Pro Kit for Feces has been optimized to process 250 mg of sample. Larger sample amount (up to 450-500 mg) can be processed with the kit to obtain higher yield (+30-40%). However, this may affect the eluent purity depending on sample complexity.
- ❑ The samples can be transferred from tube to tube by pouring during the homogenization and binding procedure to reduce plastic waste. Alternatively, pipette tips can be used to maximise recovery.

5. Safety Precautions

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 Buffer SF2 contains components that may cause irritation when in contact with human tissue. Buffer SF3 and Buffer SF4 include chaotropic salts, which can form highly reactive compounds when combined with bleach. Buffer SF4 and Buffer SF5 are flammable.

6. Protocol

1. Column SF preparation

- Add **200 µL of Equilibration Buffer** to the Column SF membranes to ensure its performance.

  Wait at least **1 min** and centrifuge for **10 sec @ maximum speed**. Transfer the Column SF into a new Collection Tube (provided).

2. Sample preparation

- Weigh up to 250 mg of the feces and add it to a Lysing Matrix YB tube.

  Note: *For hard and large stool samples, such as those obtained from goat, use a mortar or a pestle to fractionate the sample into small particles prior to addition to the Lysing Matrix YB tube.*

- Add **900 µL of Buffer SF1**. Homogenize using **Fastprep® at 5 m/s for 35 sec** or **vortex at 2500-3000 rpm for 20min**.

  Note: The performance of the DNA output (yield, purity, and DNA integrity) obtained using a 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 holding the matrix tubes with your hands, which can result in reduced homogenization efficiency, inconsistent results, and reduced yields.

- Centrifuge for **2 min @ maximum speed**.

  Note: *For herbivore feces enriched in plant particles like grass and seeds, increase the centrifugation speed (≥17, 000 x g) and/or time (2-5min).*

3. Inhibitor removal

- Transfer the supernatant (~500-700µL) into 2 mL Tube (provided).

  Optional*: The matrix tube may be re-centrifuged after transfer of the supernatant, using a tip to recover any additional lysate.*

- Add **200 µL** of **Buffer SF2** to precipitate contaminants, vortex for 1 sec and centrifuge for **2 min @ maximum speed**.

4. Binding

- Transfer the supernatant (~600-800µL) into a 2mL Tube (provided).

  Optional: The tube including the pellet may be re-centrifugated for few seconds @ 15,000 g using a tip to recover any additional lysate.

- Add **1 volume** of **Buffer SF3** and vortex for 1 sec.

  Note: if the supernatant-Buffer SF3 mixture is cloudy or contains debris, centrifuge **1min @ maximum speed** prior to binding to Column SF to increase the DNA purity. Transfer the mixture to a new 2 mL Tube (not provided)

- Apply ~**750 uL** of the lysate to the Column SF, **centrifuge for 10 sec @ 15,000 x g** and discard the flow-through. Transfer the remaining mixture to the column.

- Repeat the process until all the lysate has passed through.

  Optional: *a short spin may be performed to recover the mixtures found on the tubing lid and wall using a tip.*

5. 1st Wash

- Transfer the Column SF into a new Collection tube (provided). Add **700 µL** of **Buffer SF4** to the center of the column, **centrifuge for 10 sec @ 15,000 x g**. Discard the flow-through and place Column SF back into the same Collection tube.

6. 2nd Wash

- Add **700 µL of Buffer SF5** to the center of the column and **centrifuge for 30 sec @ 15,000 x g.**

7. Drying

- Transfer Column SF into a new Collection tube (provided), **centrifuge for 1 min @ maximum speed**.

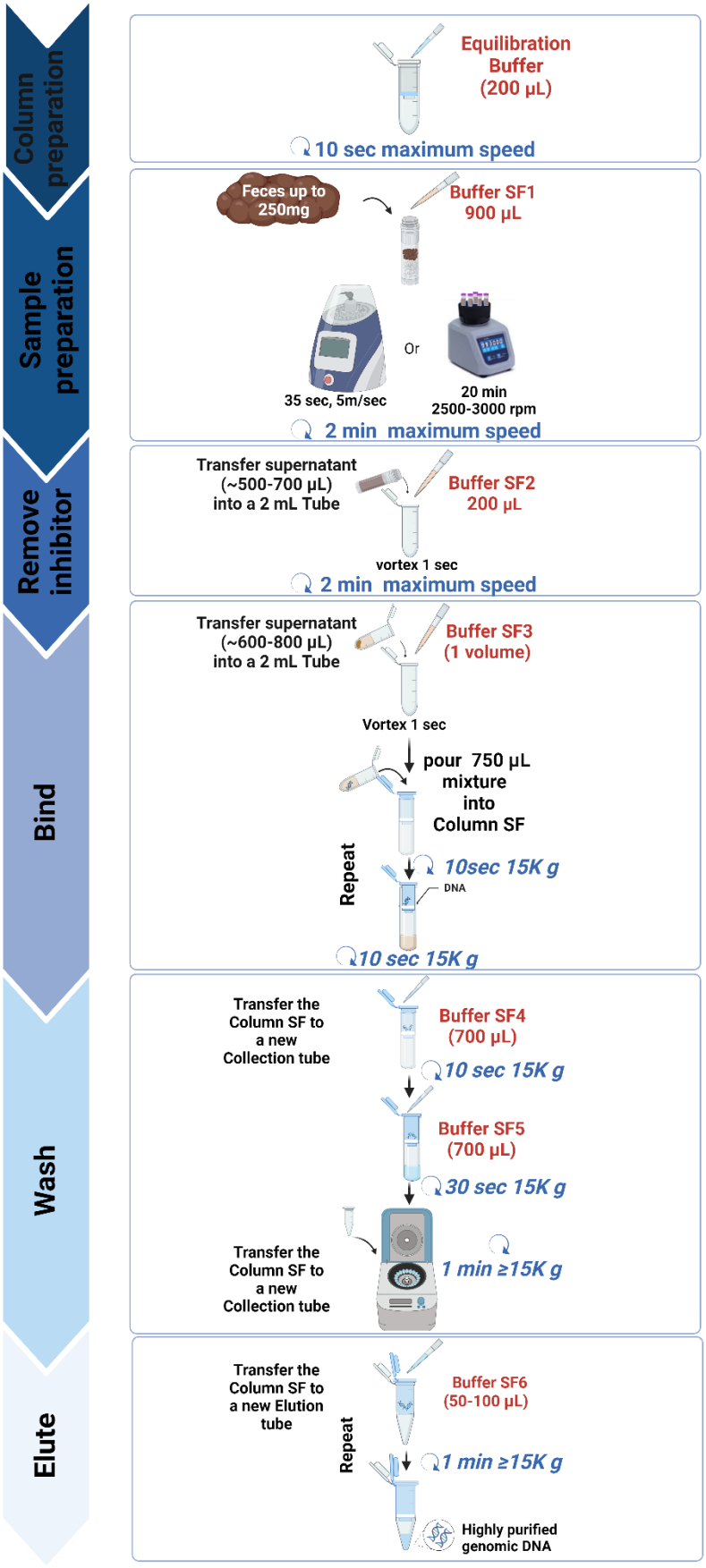
8. Elution

- Transfer the Column SF into a new Elution tube (provided). Add **100 µL of Buffer SF6** directly to the column membrane, wait for at least **1 min** and centrifuge **for 1 min @ ≥15,000 g**. The DNA sample is now ready for downstream applications.

  Note: For more concentrated sample, elute with 50µL of Buffer SF6.

Note: The nucleic acid concentration of the sample is calculated from 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 and 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 260/230 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 possible presence of humic acids, but also proteins, saccharides, ethanol, salt, and other contaminants which may inhibit subsequent enzymatic reaction.

7. Flow Chart


Column preparation
Equilibration Buffer (200 µL)
10 sec maximum speed
Sample preparation
Feces up to 250mg
Buffer SF1 900 µL
Or
35 sec, 5m/sec
20 min 2500-3000 rpm
2 min maximum speed
Remove inhibitor
Transfer supernatant (~500-700 µL) into a 2 mL Tube
Buffer SF2 200 µL
vortex 1 sec
2 min maximum speed
Bind
Transfer supernatant (~600-800 µL) into a 2 mL Tube
Buffer SF3 (1 volume)
Vortex 1 sec
pour 750 µL mixture into Column SF
Repeat
10sec 15K g
DNA
10 sec 15K g
Wash
Transfer the Column SF to a new Collection tube
Buffer SF4 (700 µL)
10 sec 15K g
Buffer SF5 (700 µL)
30 sec 15K g
Transfer the Column SF to a new Collection tube
1 min ≥15K g
Elute
Transfer the Column SF to a new Elution tube
Buffer SF6 (50-100 µL)
Repeat
1 min ≥15K g
Highly purified genomic DNA

8. Data

The SPINeasy® DNA Pro Kit for Feces was extensively optimized to provide high DNA yields along with optimal A260/280 and A260/230 ratio across a wide range of fecal samples (Figure 1, **A**). When compared to a competitor Q kit, The DNA obtained using SPINeasy® DNA Pro Kit for Feces displayed better purity and integrity (Figure 1, **B**) and can be readily used in long fragment PCR or qPCR without inhibition observed (Figure 1, **C**).

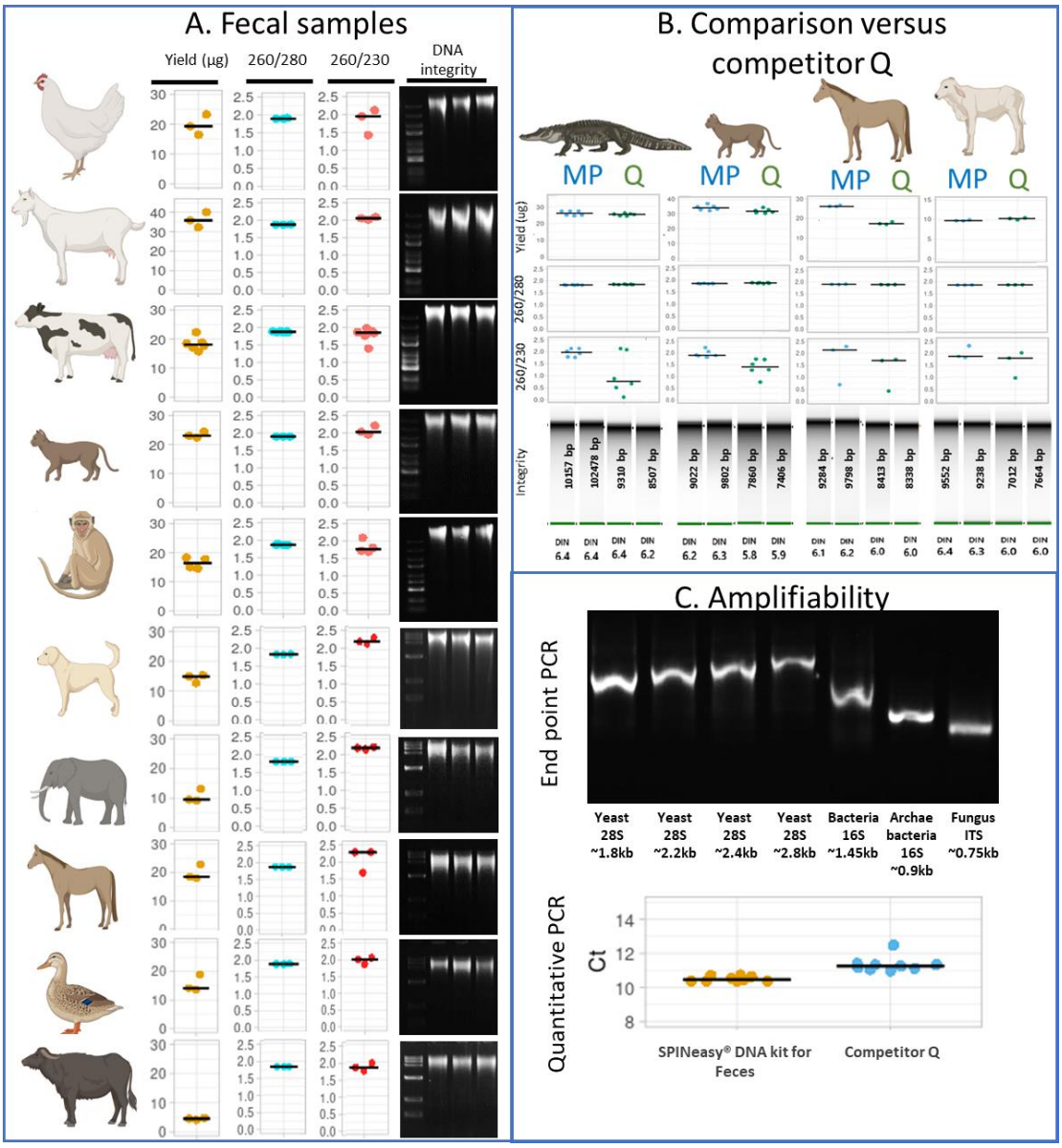


Figure 1. The SPINeasy® DNA Pro Kit for Feces provides high-performance DNA from various fecal samples. A. The SPINeasy® DNA Pro Kit for Feces kit is able to handle a wide range of samples. The DNA yield, purity (260/280 and 260/230 ratio) and integrity were assessed using spectrophotometer and DNA gel, respectively. Each dot of the plot represents a single extraction. The horizontal bars indicate the median value. **B.** Comparison with the competitor Q kit. The DNA extracted using SPINeasy® DNA Pro Kit for Feces or competitor Q kits were analyzed. Representative virtual gels obtained following Agilent 4150 TapeStation analyses were displayed along with the DNA integrity value (DIN) and the size of the genomic DNA band in bp. **C.** Amplifiability. The absence of inhibitor in fecal samples obtained using SPINeasy® DNA Pro Kit for Feces kit was assessed using inhibitor-sensitive PCR and undiluted sample as well as quantitative PCR.

9. Troubleshooting

Problem	Possible Cause	Recommendation
Wet samples		Remove the beads from the Lysing Matrix YB tubes and transfer into another clean microcentrifuge tube. Add the sample to the empty matrix YB tube and centrifuge for 30 seconds at 15,000 x g. Remove as much liquid as possible with a pipette tip. Add the beads back to the Lysing Matrix YB Tube and resume protocol from step 2.
Low DNA Yield / reduced DNA integrity	Absence of second elution with the same eluate or fresh buffer	An increase of DNA yield by ~20% can be expected by eluting twice.
	Aged sample	Fresh sample is preferred to obtain optimal yield and integrity.
	Elution using water	The elution can be performed using water, but freeze/thaw cycles may degrade DNA. It is recommended to use the elution buffer provided which is compatible with enzymatic reaction or use 5 mM Tris-HCl buffer pH 8.0.
Low A260/230 or A260/280 ratios	High level of contaminants in the sample	if the supernatant -SF3 buffer mixture is cloudy or include debris, centrifuge 1min @ maximum speed prior to binding to column SF to increase the DNA purity. Transfer the mixture to a new 2 mL Microcentrifuge tube (not provided). Perform an additional wash using Buffer SF5 (700 µL).
	Clogged column	Reduce the amount of sample.
	Particles trapped on the membrane	Debris may remain in the supernatant despite the inhibitor removal step and get loaded into the column. Those debris can be removed by inverting the column after drying and tapping the column against the bench.
	Contamination of the column's membrane	Ensure that all traces of wash buffer are removed from the column's rim prior to elution. A kleenwipe ™ or a tip connected to the vacuum manifold may be used.

High A260/280 or A260/230 ratios and low molecular weight contaminants observed in DNA gel	Possible RNA contamination	The optimized binding condition employed in the SPINeasy® DNA Pro Kit for Feces enables specific binding of gDNA and thus, the amount of RNA contaminant that is co-purified is low. These low amounts are usually not detectable using canonical detection technique and do not affect most downstream applications. However, a RNase treatment can be performed during the homogenization step by adding 25 µL of RNase A (10 mg/mL) along with Buffer SF1 prior to bead beating homogenization.
Poor PCR Performance	High concentration of DNA	Dilute the DNA 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 Pro Kit for Feces allow 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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