Instruction Manual
Research Use Only

SPINeasy DNA Pro Kit for Soil

For the isolation of genomic DNA from soil samples

Size: 50 and 5 preps
Storage: 15-25 °C
Cat. No.: 116546050 (50 preps) /
116546000 (5 preps)
Content Version: July 2022

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1. Introduction to SPINeasy DNA Pro Kit for Soil

Soil samples are complex environments characterized by the presence of inhibitory compounds, such as humic acid, heavy metals, and other aromatic components which may prove to be challenging for downstream analyses. The SPINeasy DNA Pro Kit for Soil has been carefully designed for the isolation of pure microbiome genomic DNA from challenging soil types including those with low biomass or those highly contaminated. The SPINeasy DNA Pro Kit for Soil effectively lyses various microbiome population, including bacteria, fungi, viruses, and protists. The kit provides similar yields to that of our highly cited FastDNA™ SPIN Kit, but with improved purity and reduced processing time. Isolated DNA products showed no inhibition in PCR and were immediately ready to be used for downstream applications, including long fragment PCR, qPCR, and next-generation sequencing (16S and whole genome) without the need of further inhibitor removal step.

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Kit Specifications at A Glance

Technology	Silica membrane technology
Format	Mini spin column
Vacuum manifold	Yes
Sample	Soil (e.g., sand/compost/sediments/top soil)
Sample amount	up to 500 mg
Typical yield	up to 50 µg (sample dependent)
Elution volume	30–100 µL
Preparation time	<30 min (6 preps)

2. Kit Components and User Supplied Materials

2.1 SPINeasy DNA Pro Kit for Soil Component

Product	50 reactions (Cat. No.116546050)		5 reactions (Cat. No.116546000)	
	Package	Cat. No.	Package	Cat. No.
Lysing Matrix YB	50 ea	116547051	5 ea	116547001
RNase A Solution	2 mL	116546051	200 µL	116546001
Buffer S1	55 mL	116546052	5.5 mL	116546002
Buffer S2	22 mL	116546053	2.2 mL	116546003
Buffer S3	50 mL	116546054	5 mL	116546004
Buffer S4	28 mL	116546055	3 mL	116546005
Buffer S5	40 mL	116546056	4 mL	116546006
Buffer S6	6 mL	116546057	1 mL	116546007
Column S	50 ea	116546058	5 ea	116546008
2.0 ml Collection Tubes	100 ea	116546059	10 ea	116546009
2 ml microcentrifuge tubes	100 ea	116547061	10ea	116547011
1.5 ml microcentrifuge Tubes	50 ea	116546060	5 ea	116546010
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	-

2.2 User Supplied Materials

- 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 Nuclease-free tips.
- (Optional) a commercial vacuum manifold with luer connectors connected to a vacuum pump.

3. Storage and Kit Stability

All the SPINeasy DNA Pro Kit for Soil components are guaranteed for 12 months upon reception when stored at room temperature (15-25 ˚C). For extended storage, store the Column S at 4-8˚C to maintain its performance. Storage of the Column S above 25 ˚C may decrease its performance.

4. Important Consideration Before Use

- ❑ If Buffer S1 has precipitate, heat at 37˚C until precipitate dissolves
- ❑ The SPINeasy DNA Pro Kit for Soil requires the use of a centrifuge capable of generating at least 15,000 × g to obtain optimal results.
- ❑ Shake the Buffer S2 before use.
- ❑ For faster processing, use vacuum manifold.
- ❑ The sample can be transferred from tube to tube by pouring during the homogenization and binding procedure to reduce plastic waste. Pipette tips can be also used to maximise recovery.

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. Buffer S2 contains components that may cause irritation when in contact with human tissue. Buffers S3 and S4 include chaotropic salts, which can form highly reactive compounds when combined with bleach. Buffers S4 and S5 are flammable.

6. Protocol

Weigh the soil sample **(100-500 mg)** and add it to a Lysing Matrix YB tube.

1. Homogenization.

- Add **1000 µL Lysis Buffer S1** and 25 µL of RNase A. Up to 40 µL of RNase A may be added for fresh soil with high biomass. Homogenize using a **Fastprep® at 5m/s, 35s** or **vortex for 20min @ 2500-3000rpm**, then centrifuge **for 2 mins @ ≥15,000 g.**

  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.

2. Contaminant removal.

- Pour the supernatant (~700 µL) into a 2 mL centrifuge tube (provided), tap the tube to ensure that all the supernatant is transferred without disrupting the pellet.

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

- Add **400µL Inhibitor Removal Buffer S2**, invert and mix 5 times, and centrifuge for **1 min @ ≥15,000 g**

3. Binding

- Transfer the supernatant (~1000 µL) into a 2 mL centrifuge tube (provided).

  Optional: The tube including the pellet may be re-centrifugated for 10 sec @ 15,000 g to recover any additional lysate using a tip.

- Add 900 µL Buffer S3, invert and mix twice.

  The subsequent steps can be performed using either microcentrifuge or vacuum manifold (see next page)

Microcentrifuge

- Transfer **~ 700 µL of** the previous mixture to the column S, centrifuge **for 10 sec @ 15,000 g** and discard the flow-through. Repeat the process until all the lysate has passed through the S Column.

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

Vacuum manifold

- Insert the Columns S into the vacuum manifold's luer connectors. Load **~700** µL of the lysate into the Column S by decanting and **apply vacuum**. Repeat until all the lysate has been loaded. **Switch off** the vacuum source.

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

Microcentrifuge

4. 1St Wash

- Transfer the Column S into a new 2 mL collection tube (provided). Add **500 µL of Buffer S4** to the center of the column, centrifuge **10 sec @ 15,000 g**. Discard the flow-through and place the column back into the same 2 mL Collection Tube.

5. 2nd wash

- Add **700 µL of Buffer S5** to the center of the column and centrifuge **for 1 min @ 15,000 g**.

Vacuum manifold

4. 1St Wash

- Add **500 µL of Buffer S4** to the center of the column and apply vacuum. **Switch off** the vacuum source.

5. 2nd wash

- Add **700 µL of Buffer S5** by running the pipette tip along the wall of the column and **apply vacuum**.

6. Column drying

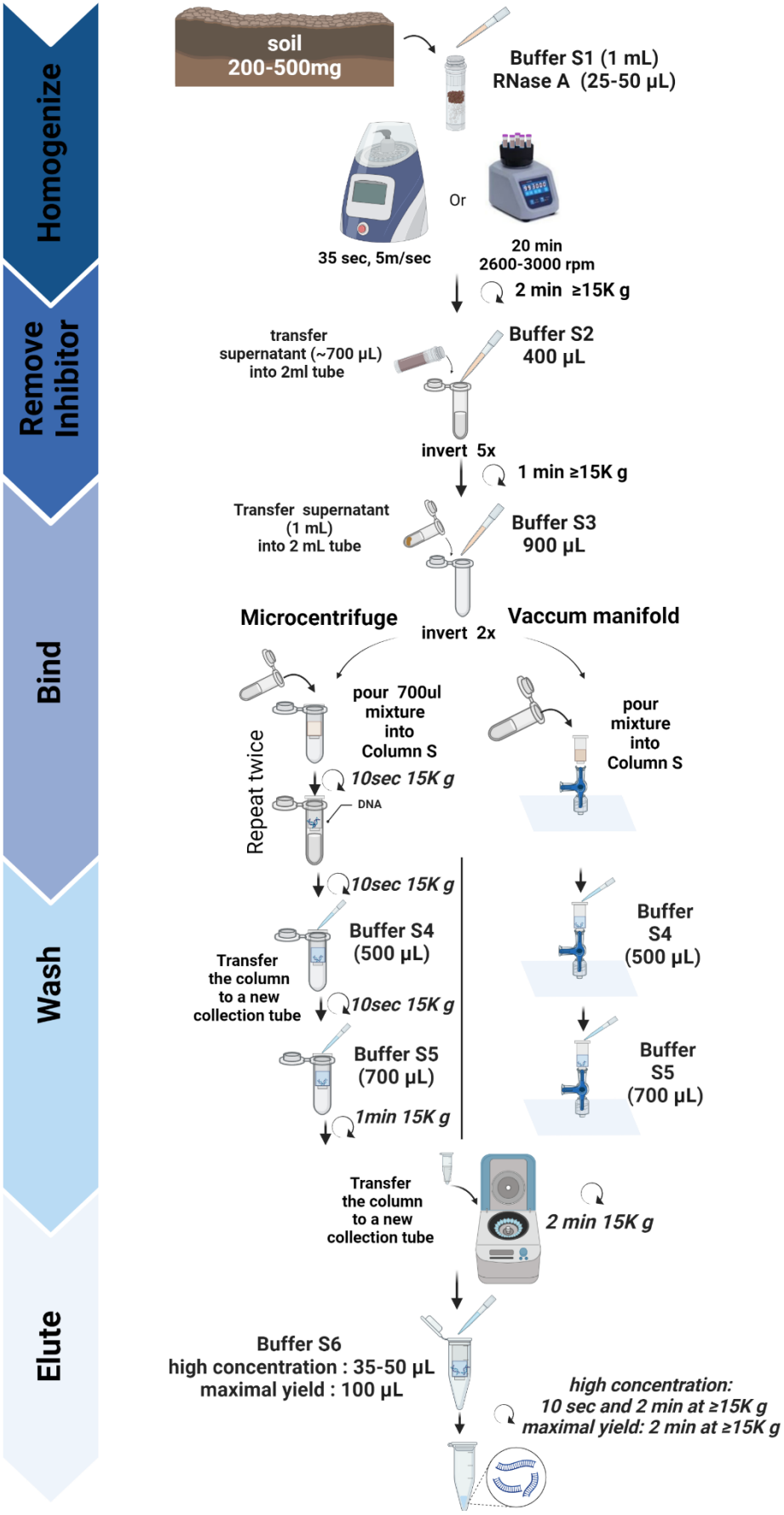
- Transfer the column into a new 2mL Collection Tube (provided), and centrifuge @ **≥15,000 g for 2 minutes**.

7. Elution

- Transfer the column into a new 1.5 mL Collection Tube (provided). To obtain maximum concentration, add **35-50 µL** of Buffer S6 to the middle of the membrane column, centrifuge @ **≥15,000 g for 10 sec** and **reload** the eluate onto the column. To achieve a maximum yield, add **100 µL of Buffer S6.** Incubate for 2-5 mins and spin for **2 mins @ ≥15,000 g**.

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 A260/A230 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, as well as proteins, saccharides, ethanol, salt, and other contaminants which may inhibit subsequent enzymatic reactions.

7. Flow Chart


Homogenize
Remove Inhibitor
Bind
Wash
Elute
soil
200-500mg
Buffer S1 (1 mL)
RNase A (25-50 µL)
Or
35 sec, 5m/sec
20 min
2600-3000 rpm
2 min ≥15K g
transfer
supernatant (~700 µL)
into 2ml tube
Buffer S2
400 µL
invert 5x
1 min ≥15K g
Transfer supernatant
(1 mL)
into 2 mL tube
Buffer S3
900 µL
invert 2x
Microcentrifuge
Vaccum manifold
pour 700ul
mixture
into
Column S
Repeat twice
10sec 15K g
DNA
pour
mixture
into
Column S
10sec 15K g
Buffer
S4
(500 µL)
Transfer
the column
to a new
collection tube
10sec 15K g
Buffer S5
(700 µL)
1min 15K g
Buffer
S4
(500 µL)
Buffer
S5
(700 µL)
Transfer
the column
to a new
collection tube
2 min 15K g
Buffer S6
high concentration : 35-50 µL
maximal yield : 100 µL
high concentration:
10 sec and 2 min at ≥15K g
maximal yield: 2 min at ≥15K g

8. Data

The SPINeasy DNA Pro Kit for Soil has been developed to accommodate a wide range of soils. The kit provides optimal yield, A260/A280 and A260/A230 ratio on fresh or frozen sample. It displays exceptional ability to extract DNA from low biomass samples (Figure 1, DNA quality).

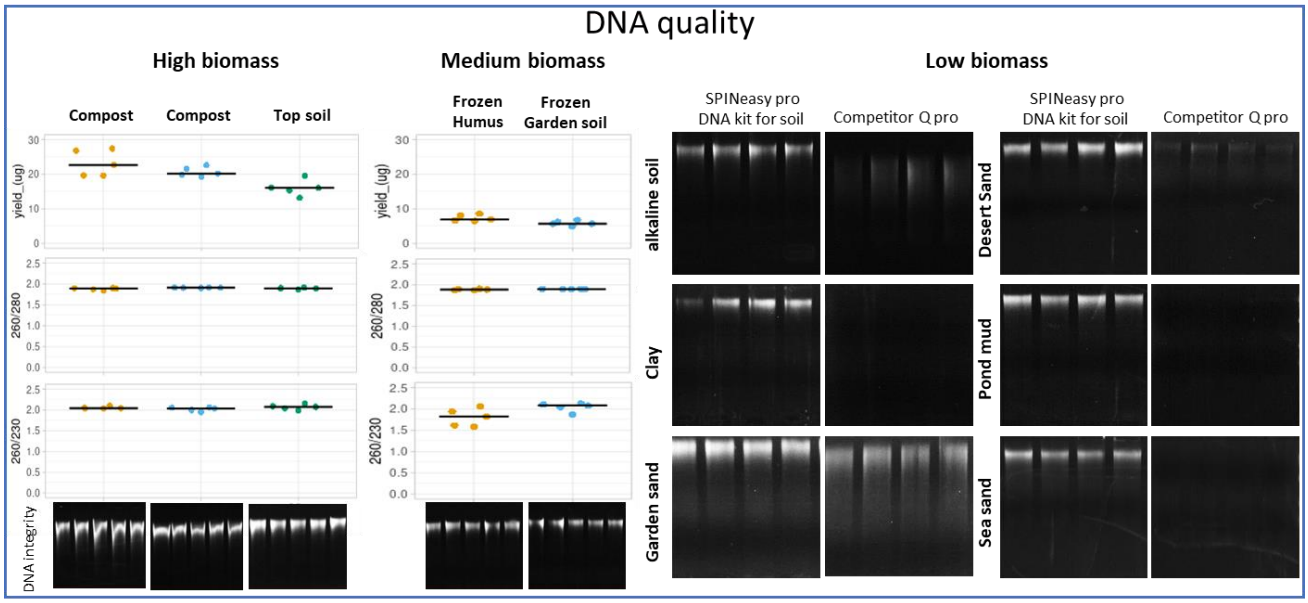


Figure 1. DNA quality. Soil sample with various biomass/ contaminant content (250 mg each) were processed with using the SPINeasy DNA Pro Kit for Soil and/or competitor kit (Q pro). The medium biomass group include samples stored at -20°C for 12-24 months which may negatively influence the yield and integrity of the isolated DNA. The DNA yield and purity (A260/A280 and A260/A230 ratio) were assessed using spectrophotometer in quadruplicate when the samples were within the detection range. Each dot of the plot represents a single extraction. The horizontal bars indicate the median value. The DNA integrity was assessed using DNA gel. For low biomass samples, similar proportion of DNA eluate were loaded to compare the performance of SPINeasy DNA Pro Kit for Soil and competitor kit.

SPINeasy DNA Pro Kit for Soil samples derived from heavily contaminated soil can be readily used in long fragment PCR and qPCR without inhibition observed (Figure 2, amplifiability). The unbiased analysis of the microbial community found in samples prepared using various methods revealed that samples extracted using the SPINeasy DNA Pro Kit for Soil are enriched in difficult to lyse gram+ bacteria such as firmicutes; this is similar to competitor Q Pro kit (Figure 2, 16s microbial analyses, left). Moreover, as compared to other extraction kits (including competitor Q Pro kit), the total number of bacteria identified, and the alpha diversity was higher (Figure 2, 16s microbial analyses, right).

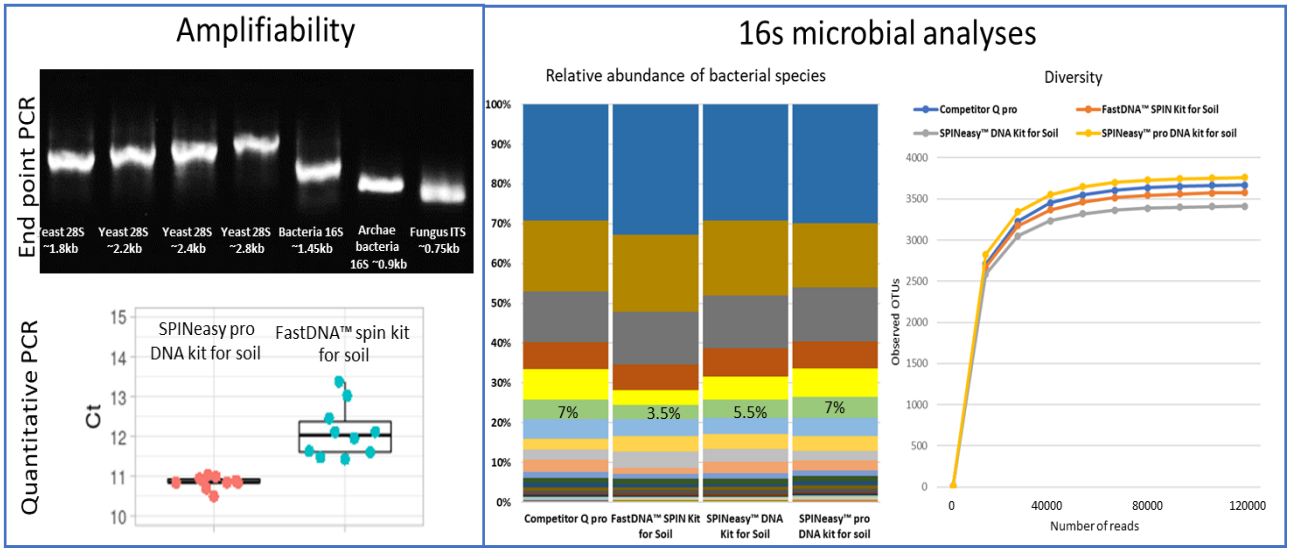


Figure 2. Amplifiability. The absence of inhibitor was assessed using inhibitor-sensitive PCR and undiluted sample as well as quantitative PCR. **16s microbial analyses.** The hypervariable *region V4* of the *bacterial 16S rRNA gene* was amplified using DNA extracted from a high biomass soil using the 4 extraction kits described in the legend. Sequences were obtained using a NovaSeq PE250 platform and analyzed using the Qiime 2 pipeline. The relative abundance of bacterial species compilated from 3 technical replicates is shown on the left. The percentage indicates the average proportion of gram positive firmicutes (yellow) found in DNA samples derived from the same soil sample. The rarefaction curves corresponding to each method are depicted on the right. The alpha diversity was measured by the number of operational taxonomic units (OTUs) identified (vertical axis) following the sequencing depth (horizontal axis).

9. Troubleshooting

Problem	Possible Cause	Recommendation
Low DNA Yield / reduced DNA integrity	Suboptimal homogenization	The time and/or speed used for FastPrep homogenization can be increased. ~15% more of genomic DNA can be expected using FastPrep when homogenized at 45 sec and 5m/sec instead of 35 sec, However, increasing DNA degradation may appear depending on the soil 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.
	Absence of second elution with the same eluate	An increase of DNA yield by ~10% can be expected by eluting twice with fresh Buffer S6.
	The sample have been stored/frozen before extraction	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/A230 or A260/A280 ratios	High level of contaminant in the sample	Increase the volume of the first wash to 700 µL or perform 2 washes using 500 µL of Buffer S4 and then a third wash using Buffer S5 (better).
	Clogged column	Reduce the amount of sample.
	Soil particles trapped on the column's membrane	Soil 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 (better) may be used.

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 Pro Kit for Soil 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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