Instruction Manual
Research Use Only

# SPINeasy DNA/RNA/Protein All-In-One Kit

For the isolation of DNA, RNA, and Protein from Single sample.

**Size:** 50 & 5 preps
**Storage:** 15-25 °C
**Cat. No.:** 116544050 (50 PREPS)
116544000 (5 PREPS)
**Content Version:** November 2023

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# 1. Introduction to SPINeasy DNA/RNA/Protein All-In-One Kit

SPINeasy DNA/RNA/Protein All-In-One Kit utilizes a convenient workflow and silica-membrane spin columns to isolate DNA, RNA and protein components from the same sample, without the use of toxic substances such as phenol and chloroform. The use of our specially formulated Lysis Buffer R and Lysing Matrix A in combination with FastPrep® Instruments from MP Biomedicals enables highly efficient lysis of tissue samples within seconds. DNA, RNA and proteins are then sequentially purified from the same lysate (Figure 1). Briefly, DNA is adsorbed onto the first spin column, while the flow-through is collected and used for RNA purification. The second spin column captures RNA and the flow-through from this step is used for protein extraction. DNA and RNA are bound on the first and second columns, respectively, and then washed and eluted. In the final extraction, proteins are precipitated out of solution, pelleted down by centrifugation, washed and resuspended. Each molecular component is then immediately available for their respective downstream applications.

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

## Kit Specifications at a Glance

| | |
|---|---|
| Technology | Silica membrane technology |
| Format | Mini spin column |
| Sample | Tissues and Cell Cultures |
| Sample amount | 30 mg for tissues, $1x10^6$ cells |
| Elution volume | 50–100 µL for DNA/RNA |

# 2. Kit Components and User Supplied Materials

## 2.1 SPINeasy DNA/RNA/Protein All-In-One Kit Component

| Components | 50 PREPS (Cat.No.: 116544050) | | 5 PREPS (Cat.No.: 116544000) | |
|---|---|---|---|---|
| | Package | Cat. No. | Package | Cat. No. |
| Equilibration Buffer R | 24 mL | 116554063 | 2.5 mL | 116554013 |
| Lysing Matrix A | 50 ea | 116910050 | 5 ea | 116910005 |
| Lysis Buffer R | 60 mL | 116541052 | 6.0 mL | 116541002 |
| Wash Buffer D | 30 mL | 116544051 | 3.0 mL | 116544001 |
| Wash Buffer R | 12 mL | 116543051 | 1.2 mL | 116543001 |
| Elution Buffer GD | 10 mL | 116532054 | 1.0 mL | 116532004 |
| Nuclease-free water | 10 mL | 116541054 | 1.0 mL | 116541004 |
| DNase I | 1 vial | 116541055 | 1 vial | 116541055 |
| DNase I Buffer | 5 mL | 116541056 | 500 µL | 116541006 |
| Protein Precipitant | 80 mL | 116544052 | 8.0 mL | 116544002 |
| Column A with collection tube | 100 ea | 116544053 | 10 ea | 116544003 |
| Quick-start protocol | 1 ea | - | 1 ea | - |
| Instruction Manual | Available www.mpbio.com | | | |
| MSDS & CoA | Available www.mpbio.com | | | |

## 2.2 User Supplied Materials

- FastPrep Instrument - FastPrep-24™ 5G (Cat. No.116005500)
- Vortex (an adapter for multiple microcentrifuge-sized tubes is recommended if multiple samples are to be processed simultaneously), if FastPrep Instrument is unavailable
- Microcentrifuge capable of at least 14,000 x g
- Absolute ethanol (at least 137.5 mL)
- 50% Ethanol (at least 25 mL)
- Nuclease-free 2 mL microcentrifuge tubes
- Nuclease-free 1.5 mL microcentrifuge tubes
- Buffer for resuspending protein pellet, such as SDS-PAGE loading buffer, 5% SDS, 50 mM NaOH/ 50 mM HCl (refer to Section 6.4)

## 3. Storage and Kit Stability

DNase I should be stored at 2-8˚C. All other components and reagents of SPINeasy DNA/RNA/Protein All-In-One Kit can be stored at room temperature (15-25℃) until the expiration date printed on the kit label. For extended storage or storage in dry condition (humidity < 40%), store the Columns at 2-8˚C to maintain performance.

## 4. Important Consideration Before Use

- ❑ Add 100 mL (10 mL for sample kit) of absolute ethanol to each bottle of Wash Buffer R and mark the bottle.
- ❑ Prepare DNase I solution according to instructions in Section 6.1.
- ❑ Prepare 750 µL of ethanol per prep for RNA binding.
- ❑ Prepare 500 µL of 50% ethanol per prep for washing of protein pellet.
- ❑ Prepare 100 – 200 µL of buffer of choice* per prep for resuspension of protein pellet. *Refer to note in Section 6.4.
- ❑ Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if 14,000 x g is not feasible.

## 5. Safety Precautions

Lysis Buffer R and Wash Buffer D contain components 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 at www.mpbio.com for additional details.

# 6. Protocol

## 1. Preparation of DNase I Solution

Briefly spin down the vial of lyophilized DNase I provided and resuspend with 500 µL of Nuclease-free water. Mix well to dissolve. Store DNase I solution at -20 °C in aliquots and avoid repeated freeze-thawing. Note: Do not prepare DNase I solution in DNase I buffer.

## 2. DNA Extraction Protocol

1. Tissues: Weigh 10 – 30 mg of tissue sample. Cut tissue into small pieces, transfer to a vial of Lysing Matrix A and add 1 mL Lysis Buffer R.
Cell culture: Resuspend cell pellet (1 x $10^6$ cells recommended) in 1 mL Lysis Buffer R and transfer to a vial of Lysing Matrix A.
2. Homogenize in a FastPrep Instrument for 15 seconds at speed setting of 4.0 m/s.
If a FastPrep Instrument is not available, lysis may be performed by vortexing samples in Lysing Matrix A at the maximum speed for 3 – 5 mins.
3. Centrifuge at 14,000 x g for 10 mins.
Note: Centrifuge at the maximum speed for all steps if 14,000 x g is not feasible.
4. Transfer the supernatant (~750 µL) to a Column A with collection tube.
5. Centrifuge at 14,000 x g for 1 min.
6. Transfer the flow-through to a clean 2 mL microcentrifuge tube and place the column back onto the collection tube. The flow-through will be used for RNA extraction (Section 6.3).
7. Add 500 µL of Wash Buffer D to the column.
8. Centrifuge at 14,000 x g for 1 min. Discard flow-through and reuse collection tube.
9. Add 500 µL of Wash Buffer R to the column. Incubate at room temperature for 1 min.
10. Centrifuge at 14,000 x g for 1 min. Discard flow-through and reuse collection tube.
11. Centrifuge at 14,000 x g for an additional 1 min to dry column.
12. Optional: Incubate at 55 °C for 3 – 5 mins to dry column completely.
13. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
14. Add 100 µL of Elution Buffer GD to the center of the membrane. Incubate at room temperature for 1 min. For samples with low DNA content, reducing the elution volume to 50 µL may increase the concentration of eluted DNA.
15. Centrifuge at 8,000 x g for 1 – 2 mins to elute DNA.

16. Eluted genomic DNA will be collected in the microcentrifuge tube. Store eluted DNA at -20 °C.

### 3. RNA Extraction Protocol

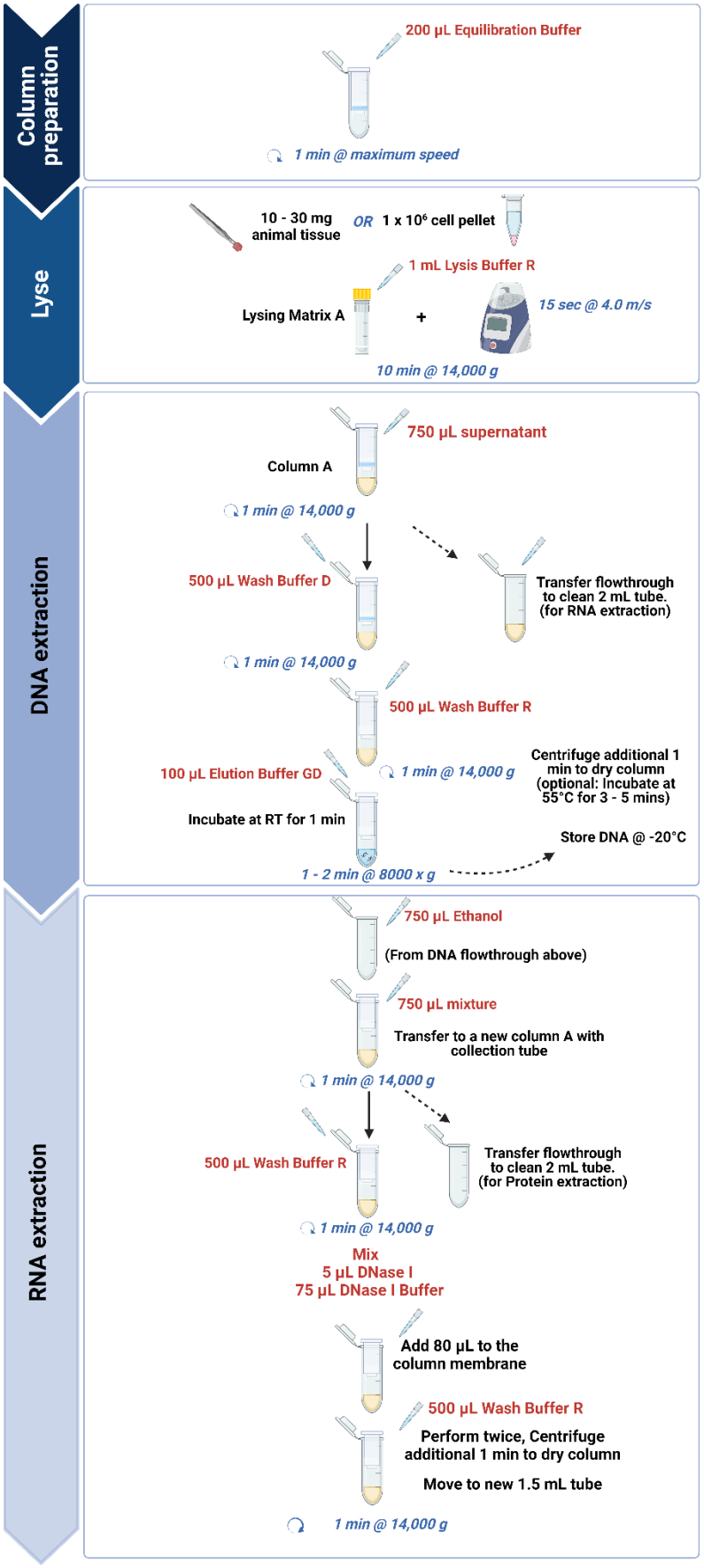
1. Add 750 µL of absolute ethanol to the flow-through collected in Step 6 of DNA Extraction Protocol (Section 6.2). Mix well by pipetting up and down.
2. Transfer 750 µL of the mixture to a new Column A with collection tube.
3. Centrifuge at 14,000 x g for 1 min.
4. Transfer the flow-through to a clean 2 mL microcentrifuge tube and place column back onto the collection tube. The flow-through will be used for protein extraction (Section 6.4).
5. Repeat steps 2 – 4 to load the remaining mixture and collect all the flow-through for protein extraction.
6. Add 500 µL of Wash Buffer R to the column.
7. Centrifuge at 14,000 x g for 1 min. Discard flow-through and reuse collection tube.
8. DNase I digestion: In a clean 1.5 mL microcentrifuge tube, add 5 µL of DNase I solution to 75 µL of DNase I Buffer per prep. Mix well and add 80 µL to the center of the column membrane. Incubate at room temperature for 15 mins.
9. Add 500 µL of Wash Buffer R to the column.
10. Centrifuge at 14,000 x g for 1 min. Discard flow-through and reuse collection tube.
11. Add 500 µL of Wash Buffer R to the column.
12. Centrifuge at 14,000 x g for 1 min. Discard flow-through and reuse collection tube.
13. Centrifuge at 14,000 x g for an additional 1 min to dry column.
14. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
15. Add 100 µL of Nuclease-free water to the center of the membrane. Incubate at room temperature for 1 min. For samples with low RNA content, reducing the elution volume to 50 µL may increase the concentration of eluted RNA.
16. Centrifuge at 8,000 x g for 1 – 2 mins to elute RNA.
17. Eluted RNA will be collected in the microcentrifuge tube. For the best results, proceed to perform downstream applications immediately and keep RNA chilled on ice while working to prevent degradation. Store remaining RNA at -80 °C in aliquots and avoid repeated freeze-thawing.

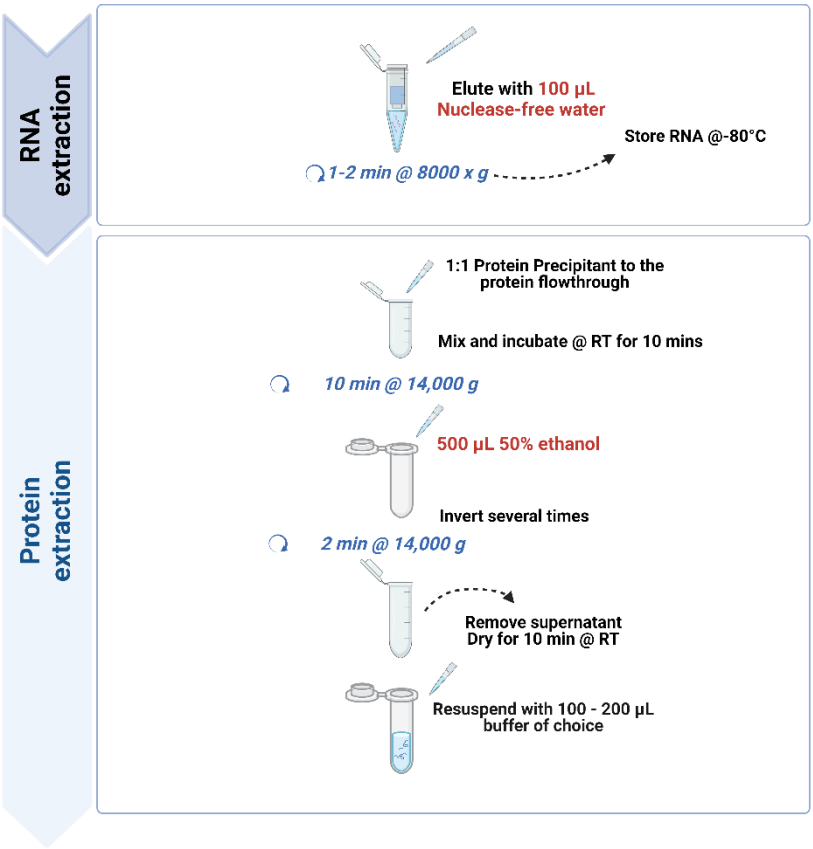
## 4. Protein Extraction Protocol

1. Add an equal volume of Protein Precipitant to the flow-through collected in Step 4 of RNA Extraction Protocol (Section 6.3).
2. Mix well by inverting tubes several times and incubate at room temperature for at least 10 minutes to allow protein precipitation.
3. Centrifuge at 14,000 x g for 10 mins. Carefully discard supernatant.
4. Add 500 µL of 50% ethanol and invert tubes several times to wash pellet.
5. Centrifuge at 14,000 x g for 2 mins.
6. Carefully remove supernatant, leaving the protein pellet in the tube.
7. Dry pellet by uncapping the tube and leaving it at room temperature for 10 minutes or longer.
8. Resuspend pellet with 100 – 200 µL of the buffer of choice*, depending on the intended downstream application.

**Note: For convenience, pellet may be dissolved in protein loading dyes, such as Laemmli buffer, and directly used for SDS-PAGE applications. Alternatively, pellet may be dissolved in 5% SDS solution. Note that SDS interferes with certain protein quantitation methods, such as the Bradford assay. Thus, buffers containing SDS, including protein loading buffers, are incompatible with such applications. If protein quantitation is necessary, the pellet may be dissolved in 50 mM NaOH, followed by neutralization with an equal volume of 50 mM HCl.*

# 7. Flow Chart


Column preparation
200 µL Equilibration Buffer
1 min @ maximum speed
Lyse
10 - 30 mg animal tissue
OR
1 x 10⁶ cell pellet
1 mL Lysis Buffer R
Lysing Matrix A
+
15 sec @ 4.0 m/s
10 min @ 14,000 g
DNA extraction
750 µL supernatant
Column A
1 min @ 14,000 g
500 µL Wash Buffer D
Transfer flowthrough to clean 2 mL tube. (for RNA extraction)
1 min @ 14,000 g
500 µL Wash Buffer R
100 µL Elution Buffer GD
1 min @ 14,000 g
Centrifuge additional 1 min to dry column (optional: Incubate at 55°C for 3 - 5 mins)
Incubate at RT for 1 min
Store DNA @ -20°C
1 - 2 min @ 8000 x g
RNA extraction
750 µL Ethanol
(From DNA flowthrough above)
750 µL mixture
Transfer to a new column A with collection tube
1 min @ 14,000 g
500 µL Wash Buffer R
Transfer flowthrough to clean 2 mL tube. (for Protein extraction)
1 min @ 14,000 g
Mix
5 µL DNase I
75 µL DNase I Buffer
Add 80 µL to the column membrane
500 µL Wash Buffer R
Perform twice, Centrifuge additional 1 min to dry column
Move to new 1.5 mL tube
1 min @ 14,000 g


RNA extraction
Elute with 100 µL
Nuclease-free water
Store RNA @-80°C
1-2 min @ 8000 x g
Protein extraction
1:1 Protein Precipitant to the protein flowthrough
Mix and incubate @ RT for 10 mins
10 min @ 14,000 g
500 µL 50% ethanol
Invert several times
2 min @ 14,000 g
Remove supernatant
Dry for 10 min @ RT
Resuspend with 100 - 200 µL
buffer of choice

## 8. Data

The following results are examples of DNA, RNA and protein extracted from animal tissue samples using SPINeasy DNA/RNA/Protein All-In-One Kit. High yields and quality of genomic DNA and RNA are obtained, as indicated by A260/A280 and A260/A230 ratios, as the expected bands clearly resolved during agarose gel electrophoresis. Extracted DNA, RNA and proteins are suitable for downstream applications, such as PCR, RT-PCR and SDS-PAGE, respectively.

**Table 1.** Quantity and quality of DNA, RNA and protein extracted from animal tissues using SPINeasy DNA/RNA/Protein All-In-One Kit.

| Sample | Starting Amount (mg) | Extraction Results | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | DNA | | | RNA | | | Protein |
| | | Yield (μg/mg) | A260/280 | A260/230 | Yield (μg/mg) | A260/280 | A260/230 | Yield (μg/mg) |
| Kidney | 17.7 | 1.28 | 1.83 | 2.42 | 1.30 | 2.04 | 2.23 | 14.12 |
| Spleen | 12.4 | 1.81 | 1.84 | 2.45 | 1.81 | 2.04 | 2.22 | 20.81 |
| Liver | 17.0 | 1.09 | 1.84 | 2.89 | 3.48 | 2.07 | 2.17 | 10.16 |

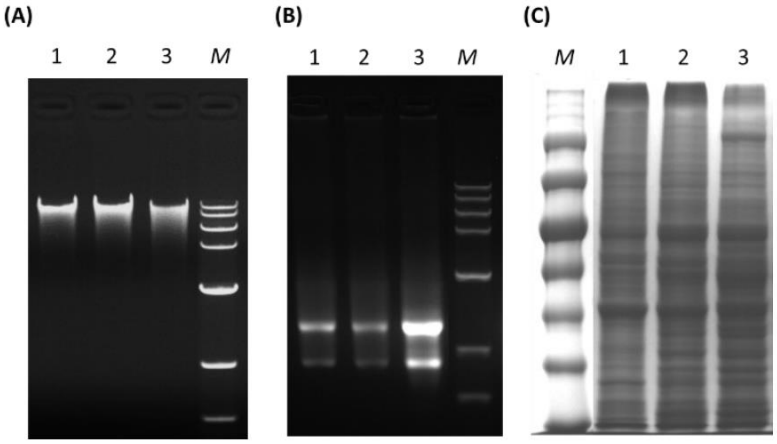



**Figure 1 (left).** (A) DNA; (B) RNA; (C) Protein extracted from each animal tissue using SPINeasy DNA/RNA/Protein All-In-One Kit, analyzed using gel electrophoresis. M: DNA Marker; Lane 1: Kidney; Lane 2: Spleen; Lane 3: Liver.

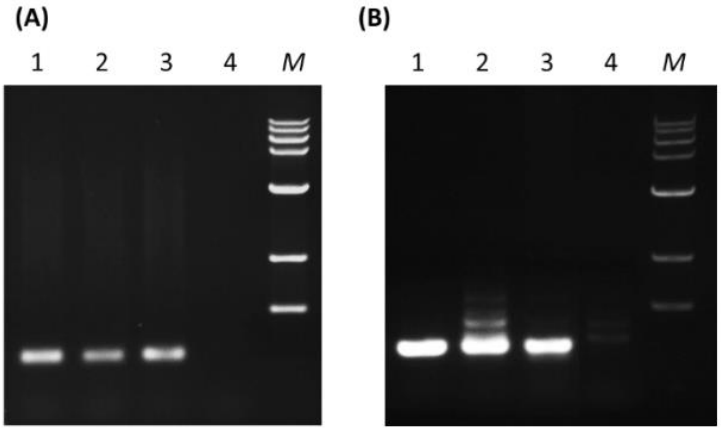



**Figure 2:** (A) PCR amplification of DNA and (B) RT-PCR amplification of RNA extracted from various samples using SPINeasy DNA/RNA/Protein All-In-One Kit. M: DNA Marker; Lane 1:Kidney; Lane 2: Spleen; Lane 3: Liver; Lane 4: Negative control.

# 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 | Recommendation |
|---|---|
| Low nucleic acid Yield / reduced integrity | Ensure the extraction was performed according to kit manual instructions. |
| | Sample with low nucleic acid content: (i) Increase amount of starting material; (ii) Elute in a smaller volume (50 µL). |
| | Insufficient lysis or over-lysis: Adjust FastPrep speed and/ or duration to achieve the optimal homogenization condition for the sample type. When using a vortex instead of a FastPrep for sample homogenization, lysis condition may be optimized by testing reduced or extended vortexing duration. |
| | Poor sample quality. For best results, freshly prepared samples should be used. |
| | Poor elution: Ensure that Elution Buffer GD or Nuclease-free water is added to the center of the column membrane. |
| | RNase contamination. Work with nuclease-free tubes and pipette tips. Handle samples and perform all steps with clean gloves. Decontaminate work surfaces with RNase Erase® (Cat. No. 112440204). |
| Smeared DNA/ RNA Bands | Poor sample quality. For best results, freshly prepared samples should be used. |
| | Sample over-lysis. Reduce FastPrep speed and/or duration. |
| | Sample degradation: Use fresh samples or freshly frozen samples. It is recommended to store samples frozen in aliquots and avoid repeated freeze-thawing. |
| | RNA degradation. Work with freshly purified RNA and keep RNA chilled on ice after elution. RNA should be stored at -80 °C; avoid multiple freeze-thaw cycles. |

| | |
|---|---|
| DNA Contamination in RNA Eluate | Perform on-column DNase I digestion according to step 8 of the RNA extraction protocol (Section 6.3). Ensure DNase I solution is prepared according to manual instructions. Once dissolved, DNase I should be stored at -20 °C. |
| No Protein Detected | Protein pellet lost. After centrifugation, protein pellet may attach loosely to the side of the tube. Decant supernatant carefully to avoid losing the pellet. |
| Protein Pellet Insoluble | As every protein has a different isoelectric point, there will be proteins that are not soluble at a certain pH. Adjust the pH of the resuspension buffer to suit the protein of interest. |
| | Increase the volume of resuspension buffer to allow more proteins to dissolve. |
| Smeared Protein Bands | Sample overloaded. Dilute protein or reduce loading volume. |
| | Interference by insoluble material. After resuspending pellet, centrifuge briefly and use supernatant. |

## 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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