Instruction Manual
Research Use Only

SPINeasy RNA Kit for Tissue (Without Lysing Matrix)

Spin Column Purification for Easy Isolation of RNA from Animal Tissue, Plant Tissue, and Cell Culture Samples

Size: 50 & 5 preps
Storage: 15-25 °C
Cat. No.: 116542050 (50 PREPS)
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1. Introduction to SPINeasy RNA Kit for Tissue (Without Lysing Matrix)

SPINeasy RNA Kit for Tissue (Without Lysing Matrix) is a silica-membrane spin-column kit that enables quick and convenient purification of total RNA from various tissue types from animals, plants and tissue cultures, without the use of toxic substances such as phenol and chloroform. With a simple workflow, this kit allows multiple samples to be processed simultaneously, producing purified RNA of high quality and integrity which are immediately ready for downstream applications such as RT-PCR.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini spin column
Sample	Animal tissue, plant tissue, and cell culture samples
Sample amount	10-30 mg (animal tissue), 50-100 mg (plant tissue), $1x10^6$ cells (cell culture samples)
Elution volume	100 µL

2. Kit Components and User Supplied Materials

2.1 SPINeasy RNA Kit for Tissue (Without Lysing Matrix) Component

Components	50 PREPS (Cat.No.: 116542050) Package	Cat. No.
Equilibration Buffer R	12 mL	112075704
Lysis Buffer R	60 mL	116541052
Wash Buffer R	12 mL	116543051
Nuclease-free water	10 mL	116541054
DNase I	1 vial	116541055
DNase I Buffer	5 mL	116541056
Column R with collection tube	50 ea	116541057
Quick-Start Protocol	1 ea	-
Instruction Manual	Available www.mpbio.com	
MSDS & CoA	Available www.mpbio.com -	

2.2 User Supplied Materials

- Mechanical lysis instrument, such as dounce homogenizer or cryogenic grinding tools (for animal and plant tissues)
- Vortex mixer with adapter
- Heat block or water bath set at 55°C (for lysis of cultured cells)
- Microcentrifuge capable of at least 14,000 *g*
- Absolute ethanol (100 mL for preparing Wash Buffer TR and 750 µL per prep for sample preparation)
- Nuclease-free 2 mL microcentrifuge tubes
- Nuclease-free 1.5 mL microcentrifuge tubes
- Single-channel pipettors (1 µL–1000 µL)
- Nuclease-free, aerosol-preventive tips
- Biohazard disposal containers
- Microcentrifuge tube rack
- Personal Protective Equipment

3. Storage and Kit Stability

DNAse I should be stored at 2-8˚C. All other components and reagents of the SPINeasy RNA Kit for Tissue (Without Lysing Matrix) can be stored at room temperature (15-25˚C) 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 Wash Buffer R and mark the bottle.
- ❑ Prepare DNase I solution according to instructions in Section 6 (No.1).
- ❑ Prepare two labelled 1.5 mL microcentrifuge tubes per prep: one for sample lysis preparation and another for elution of purified RNA.
- ❑ Prepare one 2 mL microcentrifuge tube per prep for lysate binding preparation.
- ❑ Prepare one 1.5 mL microcentrifuge tube for DNase I-buffer mixture.
- ❑ Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if 14,000 *g* is not feasible.

5. Safety Precautions

Lysis Buffer R 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 at www.mpbio.com for additional details.

6. Protocol

1. **Preparation of DNase I Solution**
   - Spin down briefly the vial of lyophilized **DNase I** provided and resuspend with **500 µL 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. **Homogenize**
   - Animal/ plant tissue: Weigh **10 - 30 mg** of animal tissue or **50 - 100 mg** of plant tissue.
   - Homogenize tissue using a mechanical lysis method, such as homogenizer or grind tissue in liquid nitrogen and transfer to a clean, nuclease-free 1.5 mL microcentrifuge tube. Add **750 µL Lysis Buffer R**. Vortex to mix.
   - Cell culture: Resuspend cell pellet (**1 x 10^6** cells recommended) in **750 µL Lysis Buffer R** and transfer to a clean, nuclease-free 1.5 mL microcentrifuge tube. Vortex for **1 min** and incubate for **10 min @ 55°C**. Vortex for **1 min** after the incubation.
   - Centrifuge samples for **5 min @ 14,000 *g***.
   - Note: Centrifuge at the maximum speed for all steps if **14,000 *g*** is not feasible.
   - Transfer lysate supernatant to a clean, nuclease-free 2 mL microcentrifuge tube.

3. **Bind**
   - Add equal volume of absolute ethanol (e.g. **750 µL** ethanol to **750 µL** lysate supernatant) and mix well by pipetting up and down.
   - Transfer **750 µL** of the mixture to a **Column R** with collection tube.
   - Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
   - Repeat to load the remaining mixture.
4. **Wash & DNase I treatment**
   - Add **500 µL Wash Buffer R** to the column.
   - Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
   - DNase I digestion:
   - In a clean 1.5 mL microcentrifuge tube, add **5 µL DNase I solution** to **75 µL 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.

5. **Wash**
   - Add **500 µL Wash Buffer R** to the column.
   - Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
   - Add **500 µL Wash Buffer R** to the column.
   - Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
   - Centrifuge for additional **1 min @ 14,000 *g*** to dry column.

6. **Elution**
   - Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
   - Add **100 µL Nuclease-free water** to the center of the membrane. Incubate for **1 min @ room temperature**. For samples with low RNA content, reducing the elution volume to **50 µL** may increase the concentration of eluted RNA.
   - Centrifuge for **1 – 2 min @ 8,000 *g*** to elute RNA.
   - 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.

7. Data

The following results are examples of RNA extracted from various animal and plant tissues using SPINeasy RNA Kit for Tissue. Lysis was performed by homogenization of samples in Lysing Matrix A using a FastPrep Instrument at speed setting of 4.0 m/s for 15 seconds. Similar results have been obtained by grinding samples in liquid nitrogen instead of FastPrep homogenization. Extracted RNA are of high quality and integrity, as indicated by A260/A280 and A260/A230 ratios of around 2, as well as 28S and 18S bands clearly resolved in agarose gel electrophoresis. RNA extracted with this kit can be amplified by RT-PCR.

Table 1: **Quality and quantity of RNA extracted from various tissues using SPINeasy RNA Kit for Tissue.**

Sample	Starting Amount (mg)	Extraction Results		
		Concentration (ng/µL)	$A_{260/280}$	$A_{260/230}$
Rabbit Liver	21.4	626.46	2.08	2.19
Rabbit Spleen	14.0	310.53	2.04	2.26
Rabbit Kidney	22.3	237.88	2.06	2.21
Rabbit Lung	21.4	159.24	2.01	2.26
Rabbit Heart	22.5	54.56	2.01	2.82
Ginkgo biloba leaf	51.3	132.93	2.09	2.21
Prunus davidiana leaf (wild peach)	52.7	382.08	2.07	1.94
Cherry leaf	48.5	294.06	2.06	1.91
Begonia leaf	52.0	368.66	2.06	1.94
Tomato leaf	52.2	663.82	2.10	2.28
Peanut leaf	70.2	898.92	2.10	2.31
Potato leaf	52.4	491.20	2.14	2.30

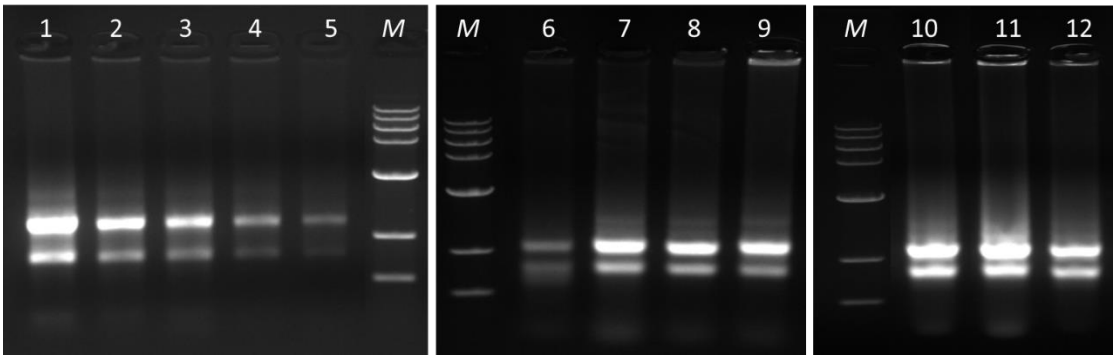

Figure 1: RNA extracted from various samples using SPINeasy RNA Kit for Tissue, analyzed using agarose gel electrophoresis. M: DNA marker; Lane 1: Liver; Lane 2: Spleen; Lane 3: Kidney; Lane 4: Lung; Lane 5: Heart; Lane 6: Ginkgo biloba leaf; Lane 7: Prunus davidiana leaf; Lane 8: Cherry leaf; Lane 9: Begonia leaf; Lane 10: Tomato leaf; Lane 11: Peanut leaf; Lane 12: Potato leaf.

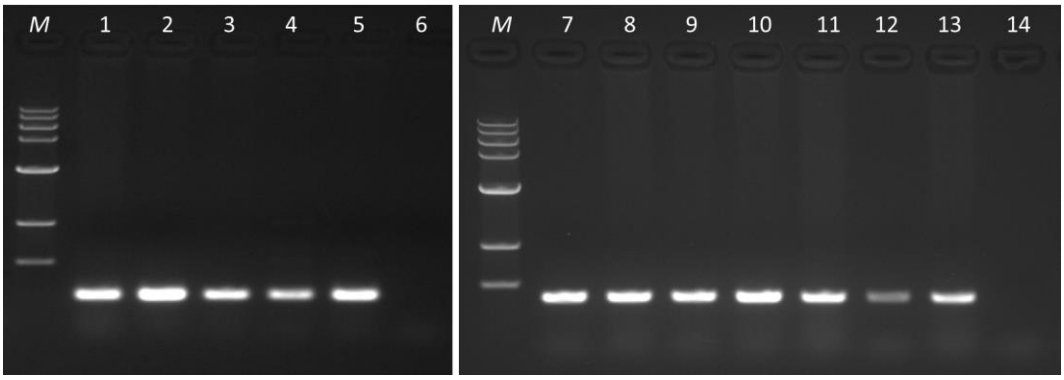

Figure 2: RT-PCR amplification of RNA extracted from various samples using SPINeasy RNA Kit for Tissue. M: DNA marker; Lane 1: Liver; Lane 2: Spleen; Lane 3: Kidney; Lane 4: Heart; Lane 5: Lung; Lane 6: Negative control (rabbit β-actin); Lane 7: Ginkgo biloba leaf; Lane 8: Prunus davidiana leaf; Lane 9: Cherry leaf; Lane 10: Begonia leaf; Lane 11: Tomato leaf; Lane 12: Peanut leaf; Lane 13: Potato leaf; Lane 14: Negative control (plant 18S).

8. 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/No RNA Yield	Inefficient extraction	Ensure the extraction was performed according to kit's manual instructions.
	Sample with low RNA content	(i) Increase amount of starting material. (ii) Elute in a smaller volume (50 µL).
	Insufficient lysis or over-lysis	Homogenization conditions may be adjusted to achieve optimal lysis efficiency for each sample type. For convenient and highly efficient homogenization, use SPINeasy RNA Kit for Tissue With Lysing Matrix (Cat#) with a FastPrep instrument (Cat. No.116005500).
	Poor sample quality	For best results, freshly prepared samples should be used.
	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 RNA bands	Poor sample quality	For best results, freshly prepared samples should be used.
	Sample over-lysis	Adjust lysis conditions.
	RNA degradation	Work with freshly purified RNA and keep RNA chilled on ice after elution. RNA should be stored at -80 °C, freeze thawing should be avoided.
DNA contamination	Inefficient DNase I digestion.	Perform on-column DNase I digestion according to step 4 of the RNA extraction protocol. Make sure that DNase I solution is prepared according to kit's manual instructions. Once dissolved, DNase I should be stored at -20 °C.

9. 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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