PRO TECH
波仕特生物科技

Gene-Spin Total RNA Purification Kit

Ver. 3/2021

For research use only

Cat#：PT-RNA-MS-50

Size：50 rxns

波仕特生物科技股份有限公司

TEL: 886-2-26557677 **FAX:** 886-2-26557601 **TEL:** 0800-231-530

E-MAIL: service@bio-protech.com.tw ; tech@bio-protech.com.tw

WEB: http://www.bio-protech.com.tw

Gene-Spin™ Total RNA Kit Flow Chart

	Animal	Plant	Cell	Bacteria
Homogenize Sample 	20-30 mg	100 mg	$5x10^6$ ~$1x10^7$	<$1x10^9$ **100 µL Lysozyme** RT, 3~10 min
RNA Lysis/2ME Solution 	**600 µL**	**450 µL** 60 ℃ , 3 min	**600 µL**	**350 µL**
Spin Lysates 	14000 x *g* 3 min	14000 x *g* 3 min	-	14000 x *g* 2 min
Lysates **+ EtOH** Mix Well 	70% EtOH **600 µL**	100% EtOH **225 µL**	70% EtOH **600 µL**	100% EtOH **250 µL**
Bind RNA 	**Load sample**		14000 x *g* 1 min	
Wash I 	**500 µL**		14000 x *g* 1 min	
DNase I Digestion 	**2 µL** DNaseI (2U/uL) **80 µL** DNaseI Buffer RT, 15 min			
Wash I 	**500 µL**		14000 x *g* 1 min	
Wash II 	1st. **600 µL**		14000 x *g* 1 min	
	2nd. **600 µL**		14000 x *g* 1 min	
Remove Residual EtOH			14000 x *g* 3 min	
Elute RNase-free H_2O 	**30~50 µL**		14000 x *g* 1 min	

Product Introduction:

Gene-Spin Total RNA Purification Kit represents a simple and convenient approach for preparation of high-purity total RNA from various **animal/plant tissues, cultured cells and bacteria**. By utilizing silica membrane column, this kit offers a variety advantages, including eliminates the requirement of ethanol precipitation and the usage of organic solvent such as phenol and chloroform. Tissues and cells are first lysed in a guanidine thiocyanate containing lysis solution by different protocols and disruption conditions. After adding ethanol, the total RNA is then bound to a miniprep column for further washing and desalting. Highly purified and full-length total cellular RNA is then eluted in a small volume of nuclease-free water and is ready for use in any downstream application. This kit could not efficiently purify the RNA which is shorter than 200 nucleotides including tRNA, 5S RNA, 5.8S RNA and microRNA.

Kit Contents:

Reagents/Tubes provided	
	Size
Spin Column with Collection Tubes	50 preps
RNA Lysis Solution	35 mL
DNase I Incubation Buffer	4.5 mL
DNase I Solution (2 U/μL)	120 μL
RNA Wash Solution I	55 mL
RNA Wash Solution II	16 mL
Nuclease-free Water	10 mL
Protocol Manual	1

DNase I Solution (cat.# PT-RNA-MS-DI-50RXN) could be purchase separately.

Storage & Stability:

Please store the **Gene-Spin Total RNA Purification Kit** at room temperature and store the DNase I solution at -20°C. All buffers are stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight or extremes in temperature.

Preparation before experiment:

1. Add 350 µl of 2-Mercaptoethanol (2-ME) to the RNA Lysis Solution, and store RNA Lysis/2-ME Solution at 4°C.
2. Add 64 ml of 100% Ethanol to the RNA Wash Solution II.

Equipment / Reagents required but not provided:

- Rotor-stator homogenizer (e.g., PolyTron). Alternatively, mortar and pestle with syringe and 20G needle.
- 2-Mercaptoethanol (2-ME): For preparing the RNA Lysis solution
- Proteinase K (only for animal fibrous tissue, e.g., heart, muscle and skin)
- Lysozyme (for bacteria strains)
- 70% Ethanol (for animal tissues and cultured cells)
- 100% Ethanol (for animal fibrous tissues, e.g., heart, muscle and skin; for RNA clean-up)
- PBS buffer (optional for attached culture cells)

Procedure:

Step1
Sample preparation **(Animal)**

Animal Tissues:

Note 1: For best quality and yield of RNA, harvest tissue immediately from a freshly sacrificed animal. Alternatively, tissue may be immediately snap-frozen in liquid nitrogen and stored at -70°C or tissue can be stabilized in RNA Keeper™ reagent (Cat# PT-R485). The sample can be stored in RNA Keeper™ reagent for a day at 37°C, 1 week at room temperature (25~28°C), one month at 4°C and indefinitely at -20°C. The purified RNA quality is as high as those stored in liquid nitrogen.

Note 2: For fibrous tissue (e.g., heart, muscle and skin), use the modified RNA isolation protocol (Appendix II), which includes a proteinase K digestion step to facilitate cell disruption.

Please use the following guidelines:

Table 1. The volume of RNA Lysis/2-ME Solution for tissue sample preparation

Sample size *1	**RNA Lysis/2-ME Solution**
<20mg tissues	350 µl or 600 µl *2
20-30 mg tissue	600 µl

*1 Do not add too much sample, or the column will be clogged, and the yield and quality would be much lower.

*2 For hard-to-lyse tissue ex: spleen, thymus, lung etc., add 600µL of RNA Lysis/2-ME Solution to less than 20mg tissue sample.

Sample preparation **(Animal)**

1. Quickly slice and weigh fresh, frozen or RNA Keeper™ reagent stabilized tissue and place into a suitable sized vessel for homogenization.

   * **IMPORTANT*** The tissue must remain frozen before adding the RNA Lysis/2-ME Solution.

2. Immediately homogenize the samples using one of the following methods:

   a. Rotor-Stator homogenization:

      Add the appropriate volume of **RNA Lysis/2-ME Solution** (see Table 1 above) to the sample, then homogenize it thoroughly until uniform without a viscous solution being formed (usually for 20~60 sec, depending on tissue type).

   b. Mortar and pestle with 20G needle and syringe homogenization:

      Freeze the sample immediately in liquid nitrogen and grind it into a fine powder under liquid nitrogen. Transfer the sample powder into a pre-chilled tube and add the appropriate volume of RNA Lysis/2-ME Solution (see Table 2 above). Homogenize the lysate thoroughly by passing it through a 20G syringe needle until uniform and without a viscous solution being formed (usually >10 times, depending on tissue type).

      ***** Mortar and pestle is used only for disruption; the lysate needs to be further homogenized and gDNA needs to be shear by passing it through a syringe needle. Otherwise, RNA yield and quality will be significantly reduced.

      ***** Do not use a Needle Gauge thinner than 20G (For example 23G), which may clog during the process of passing the lysate through.

Sample preparation **(Animal)**

3. Spin the homogenous lysate at top speed for 3 min. Carefully transfer the supernatant to a new tube without pipetting or disturbing the pellet.

   * It is important to avoid carry-over of the pellet, which may clog the column and produce much lower yield and quality.
   * If the supernatant is still viscous, the pellet may be transferred during pipetting. This indicates the homogenization is incomplete and needs to be homogenized again by a rotor-stator or 20G syringe needle.

4. Add an equal volume of **70% Ethanol** to the lysate and mix well by vortexing or pipetting. Continue to the **RNA Isolation** Section (page 13).

Sample preparation **(Plant)**

Plant Tissues:

The protocol in this manual may only work just for some plant tissues.

Note 1: For best quality and yield of RNA, harvest tissues immediately from a plant. Alternatively, tissues may be immediately snap-frozen in liquid nitrogen and stored at -70°C or can be stabilized in RNA Keeper™ reagent. The sample can be stored in RNA Keeper™ reagent for a day at 37°C, 1 week at room temperature (25~28°C), one month at 4°C and indefinitely at -20°C.

Note 2: This kit cannot be used for plant tissues with high levels of starch, phenols and secondary metabolites (such as some woody trees, milky endosperm or mycelia of filament fungi) since RNA Lysis/2-ME Solution will become solidified or very viscous after addition to sample powder.

Sample preparation **(Plant)**

1. Quickly weigh no more than 100 mg fresh, frozen or RNA Keeper™ reagent stabilized tissue and grind it into a fine powder under liquid nitrogen.
   ***** Do not allow the frozen sample to thaw during weighing before adding RNA Lysis/2-ME Solution.

2. Immediately transfer the powder (<100 mg) into a tube and add 450 µl of **RNA Lysis/2-ME Solution**, then vortex vigorously and incubate at 60°C for 3 min.
   * For samples with high starch content, omit the 60°C incubation step, otherwise the volume may increase considerably at high temperature.
3. Spin the homogenous lysate at top speed for 3 min. Carefully transfer the supernatant to a new tube without pipetting and disturbing the pellet.
   * It is important to avoid carry-over of the pellet, which may clog the column and produce much lower yield and quality.
4. Add 0.5 volumes of 100% Ethanol to the lysate and mix well by pipetting. Continue to the **RNA Isolation** Section (page 13).

Sample preparation **(Cells)**

Cultured Cells:

Table 2. The volume of RNA Lysis/2-ME Solution for cell sample preparation

Pelleted Cells	
Sample Size*	**RNA Lysis/2-ME Solution**
< 5 x 10^6	350 µl
5 x 10^6 ~ 1x 10^7	600 µl
Attached Cells	
Sample Size*	**RNA Lysis/2-ME Solution**
dish diameter < 6cm	350 µl
dish diameter < 10cm	600 µl

Table 3. Describes the number of Hela cells growing in various culture vessels when cells are grown to confluence. It may be used as a guide for estimating the number of cells.

Vessels	Growth area (cm2)	Cell number
Multi-well plate		
96-well	0.32-0.6	4.0-5.0×10^4
48-well	1	1.3×10^5
24-well	2	2.5×10^5
12-well	4	5.0×10^5
6-well	9.5	1.2×10^6
Petri Dish		
35 mm	8	1.0×10^6
60 mm	21	2.5×10^6
100 mm	56	7.0×10^6
145-150 mm	145	2.0×10^7
Bottle		
40-50 ml	25	3.0×10^6
250-300 ml	75	1.0×10^7
650-750 ml	162-175	2.0×10^7
900 ml	225	3.0×10^7

* Do not use too much sample, to prevent clogging of column, resulting in much lower yield and quality.

Sample preparation **(Cells)**

A. Suspension Cells (< 10^7 cells)

1. Pellet cells for 5 min at 300 xg and remove the medium completely.
2. Vortex or flick the tube to loosen cells and add the appropriate volume of **RNA Lysis/2-ME Solution** (see Table 2 above) to the pelleted cells.
3. Mix by vortexing or pipetting thoroughly until clump disappears.
4. Homogenize the cells for 30 sec by rotor-stator or pass the lysate through a 20G needle at least 5~10 times or until a homogenous lysate is formed, without a sticky appearance.
5. Add an equal volume of 70% Ethanol to the lysate and mix well by vortexing or pipetting. Continue to the **RNA Isolation** Section (page 13).

B. Attached Cells

Two methods can be used for sample treatment:

1. Lysing the cells directly in a culture dish

Remove medium **completely** and add the appropriate volume of **RNA Lysis/2-ME Solution** (see Table 2 above) to the dish. Immediately tip the dish to completely cover the cells, collect the lysate with a rubber policeman and follow steps (3) to (5) as per **Cells Grown in Suspension protocol**. Alternatively, without a rubber policeman, incubate for 2 min then repeat rocking the dish and transfer the lysate to a 1.5 ml microcentrifuge tube. Follow steps (3) to (5) as per **Cells Grown in Suspension protocol**.

Sample preparation **(Bacteria)**

2. Trypsinizing the cells before lysis

Remove medium **completely** and wash with PBS, then remove PBS and add 0.1%~0.25% trypsin. After the cells have detached from the dish, transfer the cells to a 1.5 ml microcentrifuge tube and centrifuge at 300 x *g* for 5 min. Completely remove the supernatant and follow steps (2) to (5) as per **Cells Grown in Suspension protocol**.

Bacteria:

Note: Before starting, prepare fresh lysozyme solution in TE buffer. Prepare 400 µg/ml for Gram-negative bacteria and 3 mg/ml for Gram-positive strain.

1. Harvest bacteria (< 1 x 10^9) by spinning at top speed for 2 min. Completely remove the medium by pipetting.
2. Vortex or flick the tube to loosen the pellet, add 100 µl freshly prepared lysozyme to the pellet and vortex to resuspend the cells.
3. Incubate the resuspended pellet at room temperature (25~28°C). For Gram-negative bacteria, incubate for 3~5 min; for Gram-positive bacteria, incubate for 5~10 min.
4. Add 350 µl **RNA Lysis/2-ME Solution** to the sample and mix by vortexing. Spin at top speed for 2 min and transfer the supernatant to a clean tube without pipetting and disturbing the pellet.
5. Add 250 µl of **100% Ethanol** to the lysate and mix well by vortex or pipetting. Continue to the **RNA Isolation** Section (page 13).

Step2
RNA
Isolation

1. Load up to 700 µl of **lysate/ethanol mixture** into an **RNA Spin Column** inserted in a 2 ml **Collection Tube**, then spin at top speed (12,000~14,000 x*g*) for 1 min and discard the flow-through.

   * If the volume of lysate/ethanol mixture is greater than 700 µl, apply any remaining lysate/ethanol mixture into the column and repeat the centrifugation step once more.

2. Transfer the RNA Spin Column into the original collection tube and add 500 µl **RNA Wash Solution I**, then spin at top speed for 1 min and discard the flow-through.

3. **DNase I** digestion**.**
   For each isolation reaction, premix 80 µl **DNase I Incubation Buffer** with 2 µl **DNase I** in a new sterile tube (Mix by flicking or inverting the tube, do not vortex!). Add 82 µl of the solution into the **center** of the RNA Spin Column membrane and incubate at room temperature (25~28°*C*) for 15 min.

   * If processing multiple samples at a time, prepare a fresh mixture of DNase I solution just before use, do not store a premix of DNase I solution.

4. Add 500 µl of **RNA wash solution I** to the RNA Spin Column, spin at top speed for 1 min and discard the flow-through.

Step2
RNA
Isolation

5. Place the RNA Spin Column to the original collection tube. Add 600 µl **RNA Wash Solution II**, spin at top speed for 1 min and discard the flow-through. Repeat this step once more.

6. Place the RNA Spin Column into the collection tube and spin at top speed for 3 min to remove any residual ethanol.

   * If the centrifugation speed is lower than 12,000 xg or residual ethanol from RNA wash solution II must be removed completely, incubate the RNA Spin Column at 60~65°C in a drying oven for 5 min to evaporate all of the remaining ethanol.

7. Place the RNA Spin Column in a clean 1.5 ml microcentrifuge tube. Add 30~50 µl of **Nuclease-free water** into the center of the RNA Spin Column membrane and let stand for 1 min. Centrifuge for 1 min at top speed to elute the RNA and store the RNA sample at -70°C.

Troubleshooting:

Problem	Solutions
Column is clogged	
1. Too much starting material	Use the correct amount of starting material. Make sure the lysate is sufficiently disrupted and homogenized.
2. Sample lysate contains insoluble residues. (Centrifugation before adding ethanol not performed)	After sample lysis (except for tissue culture cells), centrifuge the sample at top speed for 3 min or longer and only use the supernatant.
Poor RNA yield	
1. Too much starting material.	It is essential to use the appropriate amount of starting material.
2. Tissues or cultured cells are too old.	Use cultures before they reach maximum density or become fully confluent, and harvest tissues as rapidly as possible.
3. Insufficient disruption or homogenization	1. Decrease the amount of starting material. 2. Perform lysis and homogenization as recommended for each sample type using the appropriate lysis buffer as recommended. 3. Cut tissue samples into smaller pieces and homogenize them in the RNA Lysis/2-ME Solution until fully dissolved.
4. RNA still bound to the membrane	Repeat elution step, and incubate the column at room temperature for 10 min with Nuclease-free water before centrifugation.

Problem	Solutions
RNA is degraded	
1. RNase contamination	1. Use RNase-free solution, pipette tips, plastic-ware and glassware. 2. Wipe laboratory environment with RNase/ DNase/EtBR Terminator (Cat# PT-R475). 3. Change gloves frequently.
2. Improper handling of sample from harvest until lysis	1. If not processed immediately, snap-freeze tissue immediately after harvesting and store at -70°C or in liquid nitrogen. 2. Samples must remain frozen until RNA Lysis/2-ME Solution is added. 3. Tissues may be stored in RNAKeeper™ reagent
DNA contamination	
1. No DNase I treatment.	Be sure to add 2 µl DNase I (supplied with DNase I Incubation Buffer).
2. No incubation with RNA Wash Solution I before DNase I treatment.	Be sure to treat spin column with RNA Wash Solution I in step 2 before DNase I digestion.
RNA does not perform well in downstream experiments	
1. Ethanol carry-over	Be sure to dry the membrane by centrifugation at > 12,000 x *g* for 3 min. Following the centrifugation, incubate the RNA Spin Column at 60~65°C in a drying oven for 5 min to evaporate the remaining ethanol
2. Residual salt in eluate	Residual guanidine thiocyanate will also inhibit enzyme activities. Transfer the RNA Spin Column to a clean 1.5 ml microcentrifuge tube before adding RNA Wash Solution II.

Appendix I: Sample preparation from Yeast

Additional reagents required:

- Lyticase or zymolase
- Sorbitol buffer: For enzymatic lysis, prepare Sorbitol buffer containing 1 M Sorbitol, 0.1 M EDTA (pH 7.4). Add 0.1% 2-mercaptoethanol and lyticase/zymolase just before use.

1. Pellet fresh yeast cells (<5 x 10^6) at 5000 x g for 5 min at 4°C and remove the medium completely.
2. Resuspend the cells in 1 ml freshly prepared Sorbitol Buffer containing lyticase or zymolase (final concentration of 50 U per 1 x 10^7 cells). Incubate for 10~30 min at 30°C with gentle shaking to generate spheroplasts.
3. Spin down the spheroplasts at 300 x g for 5 min and carefully remove the supernatant.
4. Add 350 µl of RNA Lysis/2-ME Solution to lyse the spheroplasts and mix by vortexing vigorously. Spin at top speed for 2 min and transfer the supernatant to a clean tube without pipetting or disturbing the pellet.
5. Add 350 µl of 70% Ethanol to the lysate and mix well by vortexing or pipetting. Continue to the **RNA Isolation** Section (page 13).

Appendix II: Sample preparation from Fresh Blood Samples

Additional reagents required:

- 10X RBC Lysis Solution

1. Transfer the fresh blood sample (<0.5 ml) to the centrifuge tube and add 8 volumes of 1X RBC Lysis Solution. Mix by inverting and incubate at room temperature for 10 min or longer until red blood cells are completely lysed (solution becomes clear red); invert the tube 2~3 times during incubation.
2. Centrifuge at 3,000 x *g* for 5 min and remove the supernatant without pipetting or disturbing the pellet.
3. Resuspend and wash the leukocyte pellet in 2 volumes of 1X RBC Lysis Solution. Mix well by vortexing.
4. Centrifuge for 3,000 x *g* for 5 min and remove the supernatant without pipetting or disturbing the pellet.
5. Vortex or flick the tube to loosen the cells and add 350 µl of RNA Lysis/ 2-ME Solution.
6. Vortex or pipette thoroughly until there are no clumps and solution appears clear and homogenous.
7. Homogenize the cells for 30 sec by rotor-stator or pass the lysate through a 20G needle at least 5~10 times or until the lysate becomes homogenous, without a sticky appearance.
8. Add 350 µl of 70% Ethanol to the lysate and mix well by vortexing or pipetting. Continue to the **RNA Isolation** Section (page 13).

Appendix III: RNA Clean-Up or Genomic DNA Removal

This kit can be used to clean up RNA or to remove genomic DNA contamination for RNA samples purified using different isolation methods.

1. Adjust RNA volume to 100 µl with Nuclease-free water. Add 350 µl of **RNA Lysis/2-ME Solution** and mix well.
2. Add 250 µl of 100% Ethanol to the lysate and mix by pipetting. Continue to the **RNA Isolation** Section (page 13).

Appendix IV: Isolation of Total RNA from Heart, Muscle and Skin Tissues

Additional reagent and equipment required:

- Proteinase K (20 mg/ml)
- Water bath or heating block at 56°C

1. Process and homogenize the sample as per **Sample Preparation Section i Animal Tissue** (page 3) procedures (1) to (2). Add 590 µl Nuclease free water to the homogenate, then add 10 µl proteinase K and mix by pipetting.
2. Incubate at 56°C for 10 min.
3. Spin the lysate at top speed for 3 min and carefully transfer the supernatant to a new tube without pipetting and disturbing the pellet.
4. Add 0.5 volumes of 100% Ethanol to the lysate and mix by pipetting. Continue to the **RNA Isolation** Section (page 13).

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