Gene-Spin Human Blood RNA Purification Kit

Ver. 1/2018

For research use only

Cat#：PT-RNA-BL-50

Size：50 rxns

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Product Introduction:

Gene-Spin Human Blood RNA Purification Kit is designed for the rapid, small-scale preparation of high-purity total RNA from human blood sample. It takes the advantages of silica membrane column, which could eliminate 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 -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	
Spin Column with Collection Tubes	50 preps
Spin Filter Column with Collection Tubes	50 preps
RBC Lysis Solution	263 ml x 2
RNA Lysis Solution *[1]	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 *[2]	16 mL
Nuclease-free Water	10 mL
Protocol Manual	1

Storage & Stability:

Please store the **Gene-Spin Human Blood 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:

- 2-Mercaptoethanol (2-ME): For preparing the RNA Lysis solution
- 70% Ethanol (for lysate homogenization)
- 100% Ethanol (for preparing RNA Wash Solution II; for RNA clean-up)

Procedure:

1. Transfer the fresh whole blood sample **(0.5~1.5 ml)** to the centrifuge tube and add 5 volumes of **RBC Lysis Solution** (For Example: To 0.5 ml whole blood, add 2.5 ml RBC lysis solution). Mix by inverting and incubate at ice for 10~20 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.

   * Leukocytes will form a pellet after centrifugation. Please make sure that the RBCs are removed, otherwise the residual RBCs must affect the wash step.

3. Resuspend and wash the leukocyte pellet in 2 volumes of **RBC Lysis Solution** (For Example: To 0.5 ml whole blood, add 1 ml RBC lysis solution). Mix well by vortexing briefly.
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 **RNA Lysis/2-ME Solution**. Please refer to the table as below.

Fresh whole blood	RNA Lysis/2-ME Solution	70% Ethanol
Up to 0.5 **mL**	350 µl	350 µl
0.5 to 1.5 **mL**	600 µl	600 µl

6. Homogenize thoroughly by vortexing and pipetting until clumps disappears and the solution appears clear.

6. Immediately transfer the homogenate into a **RNA Spin Filter Column** (inserted in a clean 2 ml Collection Tube). Spin at top speed for 1 min.

   * Most cell debris can be removed by Spin Filter, but a small amount will pass through the filter and form a pellet in the collection tube. Do not disturb the pellet.

8. Transfer the flow-through from the collection tube into a new 1.5 ml microcentrifuge tube, avoiding disrupting and pipetting the pellets. Add 70% Ethanol to the flow-through and mix well by vortexing or pipetting. Please refer to the table at step 5.
9. Load up to 600 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 *xg)* for 1 min and discard the flow-through.

   * If the volume of lysate/ethanol mixture is greater than 600 μl, apply any remaining lysate/ethanol mixture into the column and repeat the centrifugation step once more.

10. 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.
11. **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 premix of DNase I solution.

12. 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.
13. 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.
14. Place the RNA Spin Column into the collection tube and spin at top speed for 5 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.

15. 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: 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 **Step 7** (page 4).

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