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PuroSPIN™ Total RNA Purification Kit

- *RNA Purification from Bacteria*
- *RNA Purification from Mammalian Cells*
- *RNA Purification from Yeast*
- *RNA Purification from Animal Tissue*
- *RNA Cleanup*
- *Genomic DNA Removal from RNA Preparation*

#NK051-50, #NK051-250

Product Manual

Version 2.2

This product is intended solely for research use only. Full material safety data sheet document is available at www.lunanano.com.

For further information and other products please refer to www.lunanano.com.

Kit Contents

Component	50 Preps (#NK051-50)	250 Preps (#NK051-250)
Lysis Buffer LB-R	40 mL	200 mL
Wash Buffer WB-R1	50 mL	225 mL
Wash Buffer WB-R2 (*after Ethanol addition*)	*60 mL*	*225 mL*
DNase I, RNase-free	550 U	5 x 550 U
DNase I Reaction Buffer	5 mL	25 mL
Elution Buffer EB	15 mL	50 mL
Water, Nuclease-free	15 mL	50 mL
PuroSPIN™ MINI Spin Columns	50	250
Collection Tubes (2 mL)	50	250

Storage

DNase I needs to be stored at ≤ **-20°C**. Once reconstituted in water, store **DNase I** at **4°C** (< 6 weeks), or in one-time-use aliquots at ≤ **-20°C.** All other components can be stored at room temperature for at least 1 year.

Additional materials and equipment required

- 2 M DTT or 14 M β-mercaptoethanol
- 96-100% Ethanol
- Pipettes, sterile nuclease-free tips
- 1.5 mL RNase-free microcentrifuge tubes
- Microcentrifuge (with 12,000g capability)
- Vortex (if not available, can be replaced with vigorous pipetting)
- Appropriate personal protective equipment (gloves, lab coat)

For RNA extraction from <u>bacterial cells</u> only:

- TE Buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), Lysozyme (3 mg/mL in TE)

For RNA extraction from <u>mammalian cells</u> only:

- PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)

For RNA extraction from <u>yeast</u> only:

- Bead-beater homogenizer (with 0.5 mm glass or zirconia beads); or **Yeast Lysis Buffer** (1 M Sorbitol, 0.1M EDTA, pH 7.4), either lyticase or zymolase enzymes

For RNA extraction from <u>animal tissue</u> only:

- Rotor-stator homogenizer, or bead-beater homogenizer (with 1-2 mm zirconia or steel beads), or mortar and pestle with liquid nitrogen

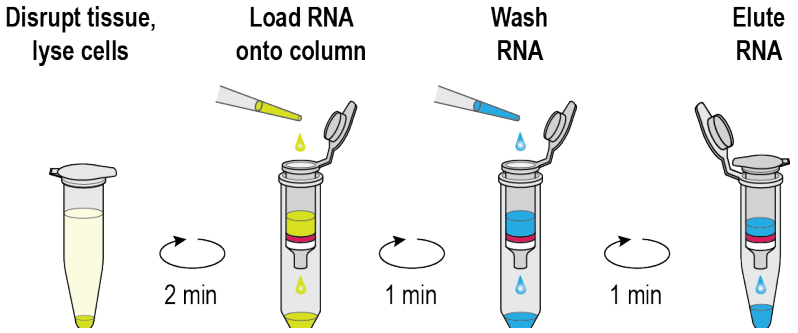
Kit Description

PuroSPIN™ Total RNA Purification Kit is designed to purify total RNA from bacteria, mammalian cells, yeast, or animal tissue. It greatly simplifies the extraction process by incorporating a convenient easy-to-use extraction technology based on nucleic acid binding silica spin-columns. The RNA is first released form the cells by the treatment with the **Lysis Buffer LB-R**. For RNA extraction from bacterial cell the lysis step is preceded by the treatment with the Lysozyme enzyme to disrupt bacterial cell wall. RNA is then loaded onto silica-based spin columns and washed to remove protein, salt and other contaminants. ***The kit includes on-column DNase I digestion step to remove any contaminating DNA***. Finally, purified RNA is eluted from the column into a clean microcentrifuge tube in **Elution Buffer EB**, or **nuclease-free water**.

The kit can also be used to perform RNA cleanup after DNase I or other enzymatic treatments. High quality RNA extracted with the **PuroSPIN™ Total RNA Purification Kit** can be used for a range of downstream applications, such as reverse-transcription PCR or qPCR, northern blotting, microarray analysis.

PuroSPIN™ Total RNA Purification Kit is capable of extracting RNA from soft animal tissue, such as liver, spleen, kidney, lung. For purification of RNA from fibrous tissue, such as skin, skeletal muscle, or heart, please use our **PuroSPIN™ Fibrous Tissue RNA Purification Kit** (#NK053-50, #NK053-250).

Experimental Workflow



Important Notes

BEFORE USE:

- Add 96-100% Ethanol to the **Wash Buffer WB-R2**:
  - **#NK051-50** (50 preps): add **45 mL** of Ethanol
  - **#NK051-250** (250 preps): add **170 mL** of Ethanol
- Reconstitute each tube of lyophilized **DNase I** in **550 µL** of nuclease-free water
  - Pulse-spin the tube before water addition to ensure the pellet is at the bottom.
  - **Do not vortex**. Vortexing might cause DNase I denaturation. Flip the tube upside down a few times to reconstitute the enzyme.
  - Store **DNase I** at **4°C** (< 6 weeks), or in one-time-use aliquots at ≤ **-20°C**.
  - #NK051-250: reconstitute one tube at a time to prevent **DNase I** degradation.

Lysis Buffer LB-R contains ***guanidine hydrochloride***. It is a known irritant that is harmful if inhaled, swallowed, or contacted with skin. Wear appropriate protective equipment when working with this reagent.

Within 30 minutes of starting RNA purification, make fresh **Working Buffer LB-R** by adding 20 µL of 2 M DTT or 14 M β-mercaptoethanol solution to each 1 mL of **Lysis Buffer LB-R** used.

If precipitate is observed in the **Lysis Buffer LB-R** or **Wash Buffer WB-R1** solutions, re-dissolve it by warming the solution to 37°C. Cool the solution back to room temperature before use.

RNA is highly prone to degradation by the RNase enzymes. RNases are very stable enzymes that are present on human skin and commonly contaminate lab equipment and surfaces. To minimize RNase contamination always were gloves when performing the extraction, use RNase-free filtered tips, and pre-treat all non-disposable equipment (pipettes, vortex, centrifuge) and surfaces with RNase removal solution. Ensure all of the buffers are tightly closed when not in use. Take particular care with the **Elution Buffer EB** and **nuclease-free water** solutions, since these are used for storage of eluted RNA.

In addition to possibility of degradation by contaminating RNase enzymes, RNA can also spontaneously break down through auto-hydrolysis. To minimize the probability of both hydrolysis and RNase degradation, the samples should either be kept on ice or frozen prior to RNA extraction. Both bacterial and mammalian cells can be pelleted and stored at -70°C until the time of RNA extraction. Lysis and homogenization steps should be performed as soon as possible after the sample is removed from ice or thawed. If fresh samples are used, the time between sample collection and RNA extraction should be minimized. Following the extraction, RNA should either be (i) kept on ice and used as soon as possible for the downstream application, or (ii) immediately frozen and stored at -70°C until needed.

<u>Protocol A</u>. Purification of Total RNA from Bacteria

RNA should be purified from bacteria harvested during the exponential growth phase (O.D. 600 measurement between 0.5 and 1). Using overnight bacterial culture can significantly decrease the amount and quality of purified RNA.

Within 30 min of starting the RNA extraction, prepare the following reagents:

- Make **Working Buffer LB-R** by adding 20 µL of 14 M β-mercaptoethanol or 2 M DTT solution to each 1 mL of **Lysis Buffer LB-R** used. Each 1 mL of **Working Buffer LB-R** is sufficient for 4 reactions.
- Make 3 mg/mL of **Lysozyme Solution** by dissolving lysozyme in the appropriate volume of TE buffer.

Step	Procedure
1	Transfer 1.5 mL of Gram-positive or Gram-negative bacterial culture (up to 10^9 CFU/mL) into 1.5 mL microcentrifuge tube. Pellet the bacteria by spinning the tube at 12,000g for 2 min. Use a pipette to carefully remove and discard as much of the supernatant as possible without disturbing the bacterial pellet.
2	To each tube add 100 µL of the **Lysozyme Solution**. Pipette up and down until homogenous suspension of cells is achieved.
3	Incubate the cell suspension for 5 min at room temperature.
4	Add 250 µL of the **Working Buffer LB-R**. Vortex vigorously for a few seconds.
5	Add 350 µL of 96-100% Ethanol. Invert a few times to mix.
6	Transfer up to 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through. *NOTE 1: If the total solution exceeds 700 µL, repeat this step to load the remaining solution onto the column.* *NOTE 2: If the liquid is still observed in the column above the filter following the 2 min centrifugation step, repeat the centrifugation until all the liquid has passed through the column. If the liquid still remains, this might be due to the column clogging by the cellular and extracellular residue. See the note on Page 15 for steps to overcome this problem.*

7	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
8	Centrifuge at 12,000g for 1 min to dry out the membrane.
9	Mix 10 µL of **DNase I** with 70 µL of **DNase I Reaction Buffer**.
10	Add 80 µL of the DNase I mixture solution to the column.
11	Incubate the column for 15 min at room temperature to allow DNA digestion to occur.
12	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
13	Add 700 µL of **Wash Buffer WB-R2** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
14	Centrifuge at 12,000g for 1 min to dry out the membrane.
15	Transfer the **PuroSPIN™ MINI Spin Column** into a new RNase-free 1.5 mL microcentrifuge tube.
16	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the RNA. Keep the flow-through that contains the product and discard the column.

Protocol B. Purification of Total RNA from Mammalian Cells

Within 30 min of starting the RNA extraction, prepare the following reagents:

- Make **Working Buffer LB-R** by adding 20 µL of 14 M β-mercaptoethanol or 2 M DTT solution to each 1 mL of **Lysis Buffer LB-R** used. Each 1 mL of **Working Buffer LB-R** is sufficient for 2 reactions.

Step	Procedure
1	a) Suspension cells Centrifuge up to 10^7 cells in an appropriate centrifuge tube at 300g for 5 min. Discard the supernatant. Wash the cell pellet with 5 mL of PBS to remove any residual growth medium. Repeat the centrifugation. Use a pipette to carefully remove and discard as much of the supernatant as possible without disturbing the cell pellet. b) Adherent cells Remove growth medium from the plate with up to $5x10^6$ adherent cells. Wash the cells with 5 mL of PBS to remove residual growth medium, discard PBS. Detach the cells by scraping in an appropriate volume of PBS or trypsinization. If the volume of cell suspension is above 1.5 mL, centrifuge cells at 300g for 5 min and resuspend in 1 mL of PBS. Transfer the cell suspension into a new RNase-free 1.5 mL microcentrifuge tube and pellet the cells by centrifugation at 300g for 5 min. Use a pipette to carefully remove and discard as much of the supernatant as possible without disturbing the cell pellet.
2	Add 500 µL of the **Working Buffer LB-R** to the pelleted cells from Step 1. Vortex vigorously for 10 seconds. *NOTE 1: If at this stage the solution becomes dense or highly viscous, homogenization of the sample is required. Homogenize the sample by using a rotor-stator homogenizer (30 seconds), or by passing the sample several times through a blunt 20-gauge RNase-free needle.* *NOTE 2: If any cell debris is observed in the sample, centrifuge the lysate for 5 min at 12,000g. Transfer the supernatant to a new RNase-free 1.5 mL microcentrifuge tube.*
3	Add 500 µL of 96-100% Ethanol. Invert a few times to mix.
4	Transfer 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through.

5	Place the **PuroSPIN™ MINI Spin Column** back into the collection tube. Transfer the remaining solution to the column. Centrifuge the column at 12,000g for 2 min, discard the flow-through.
6	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
7	Centrifuge at 12,000g for 1 min to dry out the membrane.
8	Mix 10 µL of **DNase I** with 70 µL of **DNase I Reaction Buffer**.
9	Add 80 µL of the DNase I mixture solution to the column.
10	Incubate the column for 15 min at room temperature to allow DNA digestion to occur.
11	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
12	Add 700 µL of **Wash Buffer WB-R2** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
13	Centrifuge at 12,000g for 1 min to dry out the membrane.
14	Transfer the **PuroSPIN™ MINI Spin Column** into a new RNase-free 1.5 mL microcentrifuge tube.
15	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the RNA. Keep the flow-through that contains the product and discard the column.

Protocol C. Purification of Total RNA from Yeast

Within 30 min of starting the RNA extraction, prepare the following reagents:

- Make **Working Buffer LB-R** by adding 20 µL of 14 M β-mercaptoethanol or 2 M DTT solution to each 1 mL of **Lysis Buffer LB-R** used. Each 1 mL of **Working Buffer LB-R** is sufficient for 2 reactions.
- If performing yeast cell disruption by the enzymatic lysis method (**Step 2a**), prepare **Yeast Lysis Buffer** containing 1 M sorbitol, 0.1 M EDTA, pH 7.4. Immediately before use, add β-mercaptoethanol to a final concentration of 0.1% and lyticase / zymolase enzyme in the amount of 50 U for each 10^7 yeast cells.

Step	Procedure
1	Collect up to 5 x 10^7 yeast cells by centrifuging yeast culture at 1000g for 5 min in 15 mL centrifuge tubes. Carefully discard the supernatant.
2	Disrupt the yeast cells by one of the following methods: a) Enzymatic digestion ▪ Resuspend the pellet in 1 mL of the **Yeast Lysis Buffer** supplemented with β-mercaptoethanol and lyticase or zymolase enzymes. Incubate the solution for 30 min at 30°C with gentle shaking to generate spheroplasts. Do not vortex the solution since this might disrupt the spheroplasts. ▪ Centrifuge the solution at 300g for 5 min to pellet the spheroplasts. Carefully remove and discard the supernatant. ▪ Add 400 µL of **Working Buffer LB-R** to the pellet. Vortex vigorously for 10 seconds to disrupt the spheroplasts. ▪ If insoluble material is observed in the solution, centrifuge the tube at 12,000g for 2 min. Transfer the supernatant into a new RNase-free 1.5 mL microcentrifuge tube. b) Bead-beater homogenizer ▪ Add 500 µL of the **Working Buffer LB-R** to the cells pelleted in Step 1. Vortex vigorously or pipette up and down until the pellet is resuspended. Transfer the samples into an RNase-free 2 mL screw cap tube (ensure the tube is compatible with the bead-beater apparatus). ▪ Add ~500 µL of 0.5-1 mm glass or zirconia beads into the tube using a spatula. Disrupt the tissue for 2-3 min using a bead-beater homogenizer (please refer to manufacturer's manual for specific instructions). ▪ Centrifuge the samples at 100g for 1 min to pellet the beads. Transfer the supernatant into a new RNase-free 1.5 mL microcentrifuge tube. ▪ Centrifuge the tube at 12,000g for 2 min. Transfer the supernatant into a new RNase-free 1.5 mL microcentrifuge tube.

3	Add 300 µL of 96-100% Ethanol to the solution collected in Step 2. Invert a few times to mix.
4	Transfer the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through.
5	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
6	Centrifuge at 12,000g for 1 min to dry out the membrane.
7	Mix 10 µL of **DNase I** with 70 µL of **DNase I Reaction Buffer**.
8	Add 80 µL of the DNase I mixture solution to the column.
9	Incubate the column for 15 min at room temperature to allow DNA digestion to occur.
10	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
11	Add 700 µL of **Wash Buffer WB-R2** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
12	Centrifuge at 12,000g for 1 min to dry out the membrane.
13	Transfer the **PuroSPIN™ MINI Spin Column** into a new RNase-free 1.5 mL microcentrifuge tube.
14	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the RNA. Keep the flow-through that contains the product and discard the column.

Protocol D. Purification of Total RNA from Animal Tissue

This Kit is capable of extracting RNA from soft animal tissue, such as liver, spleen, kidney, or brain. For purification of RNA from fibrous tissue, such as skin, skeletal muscle, lung, or heart, please use our PuroSPIN™ Fibrous Tissue RNA Purification Kit (#NK053-50, #NK053-250).

Within 30 min of starting the RNA extraction, prepare the following reagents:

- Make **Working Buffer LB-R** by adding 20 µL of 14 M β-mercaptoethanol or 2 M DTT solution to each 1 mL of **Lysis Buffer LB-R** used. Each 1 mL of **Working Buffer LB-R** is sufficient for 2 reactions.

Step	Procedure
1	Collect up to 30 mg of fresh or frozen tissue.
2	Homogenize the tissue using one of the following methods: a) Rotor-stator homogenizer ▪ Transfer the tissue sample into an appropriate container. Add 500 µL of **Working Buffer LB-R** into the tube. ▪ Homogenize the sample for 20-50 seconds using standard rotor-stator homogenizer (please refer to the apparatus manufacturer's manual for specific instructions). b) Bead-beater homogenizer ▪ Cut the tissue into small pieces ~1 mm in cross-section. Transfer the samples into a new RNase-free 2 mL screw cap tube (ensure the tube is compatible with the bead-beater apparatus). ▪ Add 500 µL of **Working Buffer LB-R** into the tube. ▪ Add 50-60 1-2 mm zirconia or steel beads to the sample tube using a spatula. Disrupt the tissue for 2-3 min using a bead-beater homogenizer (please refer to bead-beater apparatus manufacturer's manual for specific instructions). ▪ Centrifuge the samples at 100g for 1 min to pellet the beads. Transfer the supernatant into a new RNase-free 1.5 mL microcentrifuge tube. c) Mortar and pestle Freeze the tissue by immersing the tube containing it into liquid nitrogen. Grind the tissue thoroughly using mortar and pestle. Add 500 µL of **Working Buffer LB-R** to the tissue and disrupt it further by passing it 5-6 times through a 20-gauge needle using RNase-free syringe.

3	Transfer the homogenized mixture into a new RNase-free 1.5 mL microcentrifuge tube. Centrifuge the tube at 12,000g for 3 min. Transfer the supernatant into a new 1.5 mL microcentrifuge tube.
4	Add 300 µL of 96-100% Ethanol. Invert a few times to mix.
5	Transfer 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow-through.
6	Place the **PuroSPIN™ MINI Spin Column** back into the collection tube. Transfer the remaining solution to the column. Centrifuge the column at 12,000g for 2 min, discard the flow-through.
7	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
8	Centrifuge at 12,000g for 1 min to dry out the membrane.
9	Mix 10 µL of **DNase I** with 70 µL of **DNase I Reaction Buffer**.
10	Add 80 µL of the DNase I mixture solution to the column.
11	Incubate the column for 15 min at room temperature to allow DNA digestion to occur.
12	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
13	Add 700 µL of **Wash Buffer WB-R2** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
14	Centrifuge at 12,000g for 1 min to dry out the membrane.
15	Transfer the **PuroSPIN™ MINI Spin Column** into a new RNase-free 1.5 mL microcentrifuge tube.
16	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the RNA. Keep the flow-through that contains the product and discard the column.

Protocol E. RNA Cleanup Protocol

PuroSPIN™ Total RNA Purification Kit can be used for cleanup of RNA preparation following enzymatic reactions, such as DNase I treatment.

NOTE: For this protocol use **Lysis Buffer LB-R** directly without the addition of DTT or β-mercaptoethanol.

Step	Procedure
1	Adjust the RNA solution volume to 100 µL with **nuclease-free water**.
2	Add 250 µL of the **Lysis Buffer LB-R** (*no β –mercaptoethanol or DTT added*). Mix by vortexing or pipetting.
3	Add 350 µL of 96-100% Ethanol. Invert a few times to mix.
4	Transfer 700 µL of the solution onto the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids. Discard the flow-through.
5	Add 700 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
6	Add 700 µL of **Wash Buffer WB-R2** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
7	Centrifuge at 12,000g for 1 min to dry out the membrane.
8	Transfer the **PuroSPIN™ MINI Spin Column** into a new RNase-free 1.5 mL microcentrifuge tube.
9	Pipette 50-100 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the RNA. Keep the flow-through that contains the product and discard the column. *NOTE: Use lower elution volume to maximize the concentration of extracted RNA and larger volume to maximize total RNA recovery. To maximize total RNA recovery, repeat the elution with additional 100 µL of* ***Elution Buffer EB*** *or* ***nuclease-free water.***

Protocol F. RNA Cleanup with DNA Removal Protocol

This protocol includes a DNase I on-column digestion step designed to remove any DNA contaminating the RNA solution.

NOTE: For this protocol use **Lysis Buffer LB-R** directly without the addition of DTT or β-mercaptoethanol.

Step	Procedure
1	Adjust the volume of the RNA mixture to ~ 100 µL with **nuclease-free water**.
2	Add 250 µL of the **Lysis Buffer LB-R** (*no β –mercaptoethanol or DTT added*). Mix by vortexing or pipetting.
3	Add 350 µL of 96-100% Ethanol. Invert a few times to mix.
4	Transfer 700 µL of the solution into the **PuroSPIN™ MINI Spin Column** inserted into the 2 mL collection tube. Centrifuge the column at 12,000g for 2 min to load the nucleic acids onto the column. Discard the flow through.
5	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
6	Centrifuge at 12,000g for 1 min to dry out the membrane.
7	Mix 10 µL of **DNase I** with 70 µL of **DNase I Reaction Buffer**.
8	Add 80 µL of the DNase I mixture solution to the column.
9	Incubate the column for 15 min at room temperature to allow DNA digestion to occur.
10	Add 350 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
11	Add 700 µL of **Wash Buffer WB-R2** (*with Ethanol added*) to the column. Centrifuge at 12,000g for 1 min. Discard the flow-through.
12	Centrifuge at 12,000g for 1 min to dry out the membrane.
13	Transfer the **PuroSPIN™ MINI Spin Column** into a new RNase-free 1.5 mL microcentrifuge tube.

14	Pipette 50-100 µL of included **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000g for 1 min to elute the RNA. Keep the flow-through that contains the product and discard the column. *NOTE: Use lower elution volume to maximize the concentration of extracted RNA and larger elution volume to maximize the total RNA recovery. To maximize total RNA recovery, repeat the elution with additional 100 µL of included **Elution Buffer EB** or **nuclease-free water.***

Protocol G. Genomic DNA Removal from RNA Preparation

The protocol describes the in-solution removal of DNA from RNA preparation by **DNase I** treatment. The protocol is provided for 1 µg of RNA. It can be scaled up to the appropriate amount of RNA used.

NOTE: **10X DNase Reaction Buffer** (100 mM Tris-HCl, pH 7.5, 25 mM MgCl2, 1 mM CaCl2) is not provided with the kit.

Step	Procedure
1	Set up the following reaction: • RNA Preparation..... 1 µg • DNase I, RNase-free..... 1 unit • 10X DNase Reaction Buffer… 1 µL • RNase free water…….. to 10 µL
2	Incubate at 37°C for 30 min.
3	The reaction can be terminated either by thermal inactivation of DNase I, or by performing RNA purification. a) Inactivation Add 1 µL of 50 mM EDTA. Incubate at 65°C for 10 min. EDTA is added to chelate divalent cations to prevent RNA hydrolysis during the heating step. b) Purification Purify DNase I treated RNA using **Protocol E** of this kit.

Troubleshooting

Problem	Possible causes and solutions
Column clogging	**Too much starting material was used.** Reduce the amount of material used for the lysate preparation. Do not use higher amount of material than indicated in the protocol. **Starting material was not completely disrupted.** Perform more thorough homogenization. Use smaller amount of starting material. **Cellular debris was not completely removed.** Perform centrifugation to remove cellular debris as indicated in the protocol.
Low RNA yield	**Ethanol was not added to Wash Buffer WB2** Add the indicated amount of 96-100%. **Too much starting material was used.** Reduce the amount of material used for the lysate preparation. Do not use higher amount of material than indicated in the protocol. **Starting material was not completely disrupted.** Perform more thorough homogenization. Use smaller amount of starting material. **Ethanol was not added to the lysate.** Make sure the indicated amount of ethanol is added to the sample lysate before loading it onto the column.
Degraded RNA	**RNase contamination** Use RNase-free tips and tubes. Clean work surfaces and non-disposable items with RNase removal solution. Wear gloves. **Inappropriate handling of the sample.** When RNA is extracted from fresh samples, the samples should be kept on ice, lysis and homogenization should be performed as soon as possible. Frozen samples should be thawed immediately prior to the lysis step. Once extracted, RNA should be immediately placed on ice and stored between -20°C and -70°C.
DNA contamination	**On-column DNase digestion was not performed.** Follow the protocol steps to perform on-column DNase digestion.

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Lysis Buffer LB-R		Contains ***guanidine hydrochloride*** • Harmful if swallowed, irritating to eyes and skin. • Do not breathe vapors and fumes • Wear suitable protective clothing and gloves. • In case of contact with eyes immediately wash with plenty of water and seek medical advice. • If swallowed, seek medical advice and contact poison control center.