Luna Nanotech

PuroSPIN™ Total RNA Blood Purification Kit

Product Manual

#NK052-5	5 preps
#NK052-50	50 preps
#NK052-250	250 preps

RNA Purification from Animal and Human Blood

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Kit Description

The **PuroSPIN™ Total RNA Blood Purification Kit** is designed to purify total RNA from blood. It greatly simplifies the extraction process by incorporating a convenient easy-to-use extraction technology based on nucleic acid binding silica spin-columns. The nucleic acid is first released form the blood by the treatment with the **Lysis Buffer LB-B**. RNA is then loaded onto silica-based spin columns and washed to remove protein, salt, and other contaminants. **An on-column DNase I digestion in- between wash steps ensures the removal of any contaminating DNA**. Finally, purified RNA is eluted from the column into a clean microcentrifuge tube in **nuclease-free water.**

High quality RNA extracted with the **PuroSPIN™ Total RNA Blood Purification Kit** can be used for a range of downstream applications such as reverse-transcription qPCR, Northern blotting, or microarray analysis.

Kit Contents

Component	5 Preps (#NK052-5)	50 Preps (#NK052-50)	250 Preps (#NK052-250)
Lysis Buffer LB-B	5 mL	40 mL	200 mL
Wash Buffer WB-B1	7 mL	50 mL	225 mL
Wash Buffer WB-B2*	10 mL *	60 mL *	225 mL *
Proteinase K	150 µL	1.2 mL	5 x 1.2 mL
DNase I, RNase-free	120 U	550 U	5 × 550 U
DNase I Reaction Buffer	1 mL	5 mL	25 mL
Water, Nuclease-free	1 mL	15 mL	50 mL
PuroSPIN™ MINI Spin Columns with collection tubes	5	50	250

* After addition of 96 – 100% Ethanol

Storage

DNase I and Proteinase K should be stored at ≤ -20 °C. Once **DNase I** is re-constituted in water, store it 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

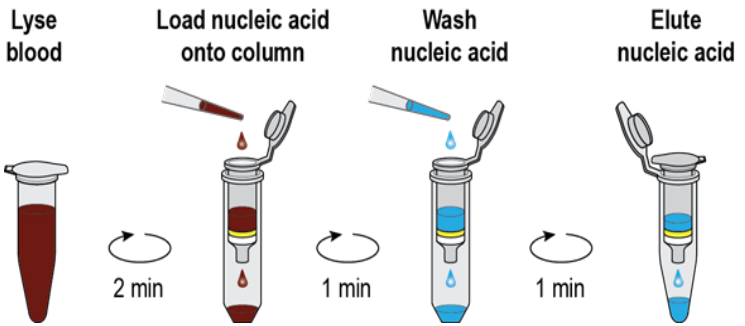
- 96 - 100% Ethanol
- Pipettes and sterile nuclease-free pipette tips
- 1.5 mL RNase-free microcentrifuge tubes
- Microcentrifuge (with 12,000 x g capability)
- Vortex (if not available, can be replaced with vigorous pipetting)
- Appropriate personal protective equipment (gloves, lab coat)

Important Notes

BEFORE USE:

- Add **96 - 100% Ethanol** to the **Wash Buffer WB-B2**:
  - **#NK052-5** (5 preps): add **7.5 mL** of Ethanol
  - **#NK052-50** (50 preps): add **45 mL** of Ethanol
  - **#NK052-250** (250 preps): add **170 mL** of Ethanol
- Reconstitute each tube of lyophilized **DNase I** in 120 µL (#NK052-5) or 550 µL (NK052-50, NK052-250) 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.
  - #NK052-250: reconstitute one tube at a time to prevent **DNase I** degradation.
- **Lysis Buffer LB-B** 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.
- If precipitate is observed in the **Lysis Buffer LB-B** or **Wash Buffer WB-B1** 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 buffer lids are tightly sealed when not in use. Take particular care with the **nuclease-free water** solution, since this is 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. 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.
- This kit can be used to purify total RNA from up to 200 µL of whole blood, serum, or plasma.

Experimental Workflow



Protocol: Purification of Total RNA from Blood

Step	Procedure
1	Add 20 µL of **Proteinase K** to the bottom of a new nuclease-free 1.5 mL microcentrifuge tube
2	Add up to 200 µL of whole blood, serum, or plasma into the tube.
3	Add 250 µL of the **Lysis Buffer LB-B**, vortex vigorously for 10 seconds
4	Incubate the tube at 50°C for 10 min.
5	Add 300 µL of **96-100% Ethanol.** Invert a few times to mix.
6	Transfer the solution into the **PuroSPIN™ MINI Spin Column** inserted into the provided 2 mL collection tube. Centrifuge the column at 12,000 x g for 2 min to load the nucleic acids onto the column. Discard the flow-through.
7	Add 350 µL of **Wash Buffer WB-B1** to the column. Centrifuge at 12,000 x g for 1 min. Discard the flow-through.
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-B1** to the column. Centrifuge at 12,000 x g for 1 min. Discard the flow-through.
12	Add 700 µL of **Wash Buffer WB-B2** (with Ethanol added) to the column. Centrifuge at 12,000 x g for 1 min. Discard the flow-through.
13	Centrifuge at 12,000 x g for 1 min to dry out the membrane.

14	Transfer the **PuroSPIN™ MINI Spin Column** into a new nuclease-free 1.5 mL microcentrifuge tube.
15	Pipette 50-100 µL of **Nuclease-free water** onto the center of the column. Centrifuge at 12,000 x g for 1 min to elute the nucleic acids. Keep the flow-through that contains the product and discard the column. *NOTE: Use lower elution volume to maximize the concentration of extracted nucleic acid or larger elution volume to maximize the total nucleic acid recovery. To maximize total nucleic acid recovery, repeat the elution with additional 100 µL of Nuclease-free water.*

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. **Starting material was not completely disrupted.** Make sure that Proteinase K was added and that 10 min 50°C incubation was carried out.
Low RNA yield	**Ethanol was not added to Wash Buffer WB-B2.** 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.** Make sure that Proteinase K was added and that 10 min 50°C incubation was carried out. **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	**Sample mixture was not fully re-suspended prior to loading onto a Silica spin column** Make sure to re-suspend the sample mixture by pipetting up and down until fully homogenized solution is achieved before loading onto a Silica spin column as failing to do so may result in contamination of purified RNA with genomic DNA. **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-B		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.

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

For further information about this and other products please visit our website at www.lunanano.com.

Learn more at lunanano.com/dna-rna-purification-products

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