Luna Nanotech

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PuroSPIN™ Viral DNA and RNA Purification Kit (96-well format)

- *Viral DNA and RNA Purification from blood, plasma, serum, milk, buccal or nasal swabs, saliva*

#NK056-480

Product Manual

Version 2.61

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.

Kit Contents

*Note: Kit comes in **2 boxes**. Ensure you have both boxes before starting.*

Component	5 x 96 Preps (#NK056-480)	
	Quantity	Location
Lysis Buffer LB-V	200 mL	Box 1
Wash Buffer WB-R1	*300 mL* *	Box 1
Wash Buffer WB-R2	*300 mL* *	Box 1
Elution Buffer EB	50 mL	Box 1
Water, Nuclease-free	50 mL	Box 1
Proteinase K (10 mg/mL), Nuclease-free	6 mL	Box 1
Carrier RNA	5 x 600 µg	Box 1
96-well Nucleic Acid Purification Plate	5	Box 1
96-well Deep Well Lysis Plates	5	Box 1
96-well Deep Well Wash Plates	5	Box 2
96-well Elution Plates	5	Box 2
Sealing Film	10	Box 2

** After addition of 96-100% Ethanol*

Storage

Carrier RNA and **Proteinase K** need to be stored at ≤ **-20°C**. Once reconstituted in water, store **Carrier RNA** 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 (preferably multi-channel), sterile nuclease-free tips
- 1.5 mL nuclease-free microcentrifuge tubes
- Appropriate vacuum manifold, or centrifuge capable of processing stacked microplates
- TE Buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) *or* PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)
- Appropriate personal protective equipment (gloves, lab coat)
- *Optional*: Water bath or heating block (heated to 55°C)

Kit Description

PuroSPIN™ Viral DNA and RNA Purification Kit is designed for extraction of viral genomic nucleic acids from blood, plasma, serum, buccal or nasal swabs, and saliva. The kit can be used both with RNA and DNA viruses as our buffers formulations and membranes are specifically optimized to bind both RNA and DNA nucleic acids. The kit greatly simplifies the extraction process by incorporating a convenient easy-to-use extraction technology based on nucleic acid binding silica spin-columns. The virus in the sample is first digested and nucleic acid released by the treatment with Proteinase K and **Lysis Buffer LB-V**. Following the lysis step, nucleic acid is loaded onto silica-based membranes and washed to remove protein, salt and other contaminants. Finally, purified nucleic acid is eluted from the membranes into a clean 96 well plate in **Elution Buffer EB**, or **nuclease-free water**. High quality DNA and RNA extracted with the **PuroSPIN™ Viral DNA and RNA Purification Kit** can be used for a range of downstream applications, such as reverse transcription or qPCR. PuroSPIN™ Nucleic Acid Purification Plates have a very high binding capacity and efficiently bind both DNA and RNA nucleic acids.

Important Notes

BEFORE USE:

- Add 96-100% Ethanol to the **Wash Buffer WB-R1**:
  - **#NK056-480** (5 x 96 preps): add **112 mL** of Ethanol.
- Add 96-100% Ethanol to the **Wash Buffer WB-R2**:
  - **#NK056-480** (5 x 96 preps): add **225 mL** of Ethanol.
- Reconstitute each tube of 600 µg of lyophilized **Carrier RNA** in 600 µL of **Elution Buffer EB** to make 1 µg/µL solution.
  - Pulse-spin the tube before buffer addition to ensure the pellet is at the bottom.
  - Immediately after reconstitution aliquot the **Carrier RNA** solution into 50 µL aliquots. Store the aliquots at ≤ **-20°C**.
  - When working with Carrier RNA, keep the solution on ice to prevent RNA degradation. Avoid multiple freeze-thaw cycles of the solution aliquots as this might lead to RNA degradation.
  - Reconstitute one tube at a time to prevent **Carrier RNA** degradation.

Lysis Buffer LB-V and **Wash Buffer WB-R1** contains ***guanidine salts***. These are known irritants that are 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-V** or **Wash Buffer WB-R1**, 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. Therefore, when extracting viral RNA always were gloves, 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.

While viral DNA is generally much more stable compared to viral RNA, when extracting the nucleic acid from DNA viruses, it is still recommended to use DNase-free filtered tips and keep sample on ice after extraction.

All sample material and waste should be regarded as potentially infectious. Please wear proper protective equipment when handling samples and waste solutions and follow proper procedures for handling biohazard materials. Work in biosafety cabinet or similar laminar air flow cabinet if possible until samples are lysed. Disinfect all work surfaces thoroughly after the procedure. Follow proper waste disposal guidelines as recommended by local authorities.

Carrier RNA (included with the kit) serves an important role in the purification of viral nucleic acids. First, viral nucleic acids can be present at very low copy number in the sample. At such low concentrations the ability of DNA or RNA to bind to the silica membrane is significantly reduced. However, addition of Carrier RNA enhances the binding of low copy nucleic acids to the membrane, greatly increasing the yield of the purified product. Second, in the case of small contamination of the sample with RNase enzymes, more abundant Carrier RNA preferentially acts as the RNase enzyme substrate, preventing RNase binding and degrading of the viral RNA.

Proteinase K (included with the kit) aids in the digestion of viral proteins which can improve yield from certain samples. Viral proteins can make up structural components of the particle or may form nucleo-protein complexes with the genetic material of the virus. We include Proteinase K with our viral kits to aid in the digestion of these problematic proteins and fully break down the virus. The reaction is performed at room temperature for 10 minutes. However, for some difficult to digest viral sources, elevated temperatures may be required.

For difficult to digest samples, the plates can be sealed with a provided Sealing Film and incubated at temperatures up to 55°C for 10 minutes. If condensation forms on the Sealing Film, briefly spin the plates in a centrifuge to collect the condensate before opening.

Protocol. Purification of Viral DNA and RNA

Within 30 min of starting the nucleic acid extraction, prepare the following reagents. Keep **Working Buffer LB-V** on ice to prevent **Carrier RNA** degradation.

- Make **Working Buffer LB-V** by:
  - Adding 5 µL of 1 µg/µL of **Carrier RNA** solution to each 300 µL of **Lysis Buffer LB-V** used.
  - Adding 10 µL of 10mg/ml **Proteinase K** solution to each 300 µL of **Lysis Buffer LB-V** used.
- Calculate the total amount of **Working Buffer LB-V** needed as follows:
  - Volume of **Working Buffer LB-V** needed = (300 µL) x (number of samples)

A. Extraction Protocol for Centrifuges

Step	Procedure
1	Sample preparation: a) Purification from samples in Viral Transport Medium (VTM) ▪ Pulse centrifuge VTM tubes containing sample, then gently vortex to form a uniform suspension. ▪ Use 200 µL of the sample. b) Purification from blood, serum, plasma ▪ Use 200 µL of EDTA or citrate treated blood, serum or plasma. c) Purification from milk ▪ Use 200 µL of milk sample. d) Purification from fresh nasal or buccal swabs ▪ Swirl the swab for 2-4 minutes in 200 µL of TE or PBS buffer to transfer the cells and viral particles to the buffer. Use the buffer for Step 3. e) Purification from dried nasal or buccal swabs ▪ Break the swab tip into the mixture of 300 µL of **Working Buffer LB-V** and 200 µL of PBS in a well of fresh **Deep Well Lysis Plate**. ▪ Seal the plate with a provided Sealing Film and incubate* for 10 min at 25°C. ▪ Remove the Sealing Film and carefully take out the swab tip and move directly to Step 5. f) Purification from saliva ▪ Centrifuge saliva sample at 4,000 x g for 5 min. Discard the supernatant. ▪ Resuspend the pelleted cells in 200 µL of TE or PBS buffer. Use the buffer for Step 3.

2	Add 300 µL of **Working Buffer LB-V** to each well of one of the **Deep Well Lysis Plates**.
3	Transfer 200 µL of sample or resuspension buffer from Step 1 to a well of the **Deep Well Lysis Plate** containing **Working Buffer LB-V**.
4	Mix thoroughly by pipetting. Seal the plate with a provided Sealing Film. Incubate* the samples for 10 min at 25°C. Remove the Sealing Film.
5	Add 300 µL of 96-100% Ethanol to each well. Mix thoroughly by pipetting.
6	Transfer 800 µL of the solution onto the **PuroSPIN™ Nucleic Acid Purification Plate** placed above a *fresh* **Deep Well Wash Plate**. Centrifuge the stacked plates at 3,000 x g for 2 min to load the nucleic acids onto the membrane. Discard the flow-through from the lower plate and tap the wash plate upside-down on a stack of clean paper towels to remove excess droplets. Reassemble.
7	Add 500 µL of **Wash Buffer WB-R1** (*with Ethanol added*) to each sample of the plate. Centrifuge at 3,000 x g for 2 min. Discard the flow-through from the lower plate and tap the wash plate upside-down on a stack of clean paper towels to remove excess droplets. Reassemble.
8	Add 500 µL of **Wash Buffer WB-R2** (*with Ethanol added*) to each sample of the plate. Centrifuge at 3,000 x g for 2 min. Discard the flow-through from the lower plate and tap the wash plate upside-down on a stack of clean paper towels to remove excess droplets. Reassemble.
9	Centrifuge at 3,000 x g for 10 min to dry out the membrane.
10	Move the **PuroSPIN™ Nucleic Acid Purification Plate** onto a fresh **96-well Elution Plate**.
11	Pipette 100 µL of **Elution Buffer EB** or **nuclease-free water** onto the ***center*** of the membrane of each well. Centrifuge at 3,000 x g for 2 min to elute the DNA and RNA. Keep the flow-through that contains the product.

*** NOTE: (Step 1e and Step 4):** For digestion of resistant samples, seal the plate with an included Sealing Film and incubate at an elevated temperature, up to 55°C. Then briefly spin the plate in a centrifuge to remove any condensation before removing the Sealing Film. Continue with the protocol.

B. Extraction Protocol for Vacuum Manifolds

Note:

Individual manifolds vary in their specifications. Check with your manifold manufacturer for specific usage instructions.

Processing samples with vacuum manifold is a faster protocol than centrifugation and will produce high yields of high-quality nucleic acids, suitable for many downstream applications. However, buffers can remain on the column and impact your application's performance. It is critical to adhere to the steps below for best results.

Step	Procedure
1	Sample preparation: a) Purification from samples in Viral Transport Medium (VTM) ▪ Pulse centrifuge VTM tubes containing sample, then gently vortex to form a uniform suspension. ▪ Use 200 µL of the sample. b) Purification from blood, serum, plasma ▪ Use 200 µL of EDTA or citrate treated blood, serum or plasma. c) Purification from milk ▪ Use 200 µL of milk sample. d) Purification from fresh nasal or buccal swabs ▪ Swirl the swab for 2-4 minutes in 200 µL of TE or PBS buffer to transfer the cells and viral particles to the buffer. Use the buffer for Step 3. e) Purification from dried nasal or buccal swabs ▪ Break the swab tip into the mixture of 300 µL of **Working Buffer LB-V** and 200 µL of PBS in a well of fresh **Deep Well Lysis Plate**. ▪ Seal the plate with a provided Sealing Film and incubate* for 10 min at 25°C. ▪ Remove the Sealing Film and carefully take out the swab tip and move directly to Step 5. f) Purification from saliva ▪ Centrifuge saliva sample at 4,000 x g for 5 min. Discard the supernatant. ▪ Resuspend the pelleted cells in 200 µL of TE or PBS buffer. Use the buffer for Step 3.
2	Add 300 µL of **Working Buffer LB-V** to each well of one of the **Deep Well Lysis Plates**.

3	Transfer 200 µL of sample or resuspension buffer from Step 1 to a well of the **Deep Well Lysis Plate** containing **Working Buffer LB-V**.
4	Mix thoroughly by pipetting. Seal the plate with a provided Sealing Film. Incubate* the samples for 10 min at 25°C. Remove the Sealing Film.
5	Add 300 µL of 96-100% Ethanol to each well. Mix thoroughly by pipetting.
6a	Assemble your vacuum manifold with the **PuroSPIN™ Nucleic Acid Purification Plate** placed above the waste tray, ***or*** if no waste tray is provided by your manufacturer, a *fresh* **Deep Well Wash Plate** can be used in the waste tray's place.
6b	Transfer 800 µL of the solution onto the **PuroSPIN™ Nucleic Acid Purification Plate.** Apply vacuum to load the nucleic acids onto the membrane. Aim for 1-2 drops per second. Release vacuum slowly. If needed, discard the flow-through from the lower plate and tap plate upside-down on a stack of clean paper towels to remove excess droplets. Reassemble.
7	Add 500 µL of **Wash Buffer WB-R1** (*with Ethanol added*) to each sample of the plate. Apply vacuum to wash the membrane. Aim for 1-2 drops per second. Release vacuum slowly. If needed, discard the flow-through from the lower plate and tap plate upside-down on a stack of clean paper towels to remove excess droplets. Reassemble.
8	Add 500 µL of **Wash Buffer WB-R2** (*with Ethanol added*) to each sample of the plate. Apply vacuum to wash the membrane. Aim for 1-2 drops per second. If lower plate is full, release vacuum slowly and discard the flow-through from the lower plate and tap plate upside-down on a stack of clean paper towels to remove excess droplets. Reassemble.
9a	Apply vacuum for an additional 10 min to dry out the membrane. Release vacuum slowly.
9b	Remove the **PuroSPIN™ Nucleic Acid Purification Plate** from the manifold. Remove any residual liquid and drops from the bottom of the plate, either by wicking, or by tapping plate bottom on a stack of clean laboratory wipes or paper towels.
10	Reassemble the vacuum manifold to position the **PuroSPIN™ Nucleic Acid Purification Plate** above a fresh **96-well Elution Plate**.

11	Pipette 100 µL of **Elution Buffer EB** or **nuclease-free water** onto the ***center*** of the membrane in each well. Briefly apply vacuum to pull the liquid into the membrane. Wait 30 s, then apply vacuum to elute the DNA and RNA. ***Carefully*** release the vacuum**.** Keep the flow-through that contains the product.

*** NOTE: (Step 1e and Step 4):** For digestion of resistant samples, seal the plate with an included Sealing Film and incubate at an elevated temperature, up to 55°C. Then briefly spin the plate in a centrifuge to remove any condensation before removing the Sealing Film. Continue with the protocol.

Troubleshooting

Problem	Possible causes and solutions
Membrane clogging	**Starting material was not completely disrupted.** Perform more thorough homogenization. Try a longer digestion with Proteinase K at an elevated temperature. (See the note at the bottom of Page 5). Try using less starting material. **Precipitates were not completely removed.** When using plasma samples, remove visible kryoprecipitates by centrifugation for 3,000 x g for 5 min.
Low nucleic acid yield	**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 membrane. **Ethanol was not added to Wash Buffers.** 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. **Carrier RNA was not added to the lysate.** Add the indicated amount to create the working buffer before starting each extraction. **Degraded Carrier RNA.** Avoid multiple cycles of freeze-thawing of reconstituted Carrier RNA. Always keep thawed Carrier RNA on ice. Store frozen aliquots of Carrier RNA below -20°C **Viral nucleic acid eluate too dilute.** Try Elution with just 50 µL of Elution Buffer EB. **Starting material was not completely disrupted.** Perform more thorough homogenization. Try a longer digestion with Proteinase K at an elevated temperature. (See note at the bottom of Page 5). Use less starting material. **Low elution volume (Vacuum Processing)** Ensure elution buffer was added to center of silica membrane, and not on the walls of the well or O-ring.
Degraded RNA	**RNase contamination** Use RNase-free tips and tubes. Clean surfaces, tools, and equipment 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 below -20°C.

Inhibited downstream reactions	**Residual Wash Buffers** Follow wicking/drying steps carefully. Perform step 8 twice (*i.e.* Wash twice with WB-R2) Dilute eluent 2x with Elution Buffer or nuclease free water. Consider centrifugal processing steps over vacuum processing. **RNase contamination** Use RNase-free tips and tubes. Clean surfaces, tools, and equipment 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 below -20°C.

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Lysis Buffer LB-V		Contains ***guanidine thiocyanate*** • 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.
Wash Buffer WB-R1		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.