Luna Nanotech

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PuroSPIN™ Viral DNA and RNA Purification Kit

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

#NK055-50, #NK055-250

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

Kit Contents

Component	50 Preps (#NK055-50)	250 Preps (#NK055-250)
Lysis Buffer LB-V	20 mL	100 mL
Wash Buffer WB-R1	60 mL	225 mL
Wash Buffer WB-R2	*60 mL* *	*225 mL* *
Elution Buffer EB	15 mL	50 mL
Water, Nuclease-free	15 mL	50 mL
Proteinase K (10 mg/mL), Nuclease-free	600 µL	3 x 1 mL
Carrier RNA	300 µg	3 x 600 µg
PuroSPIN™ MINI Spin Columns	50	250
Collection Tubes (2 mL)	50	250

* *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.

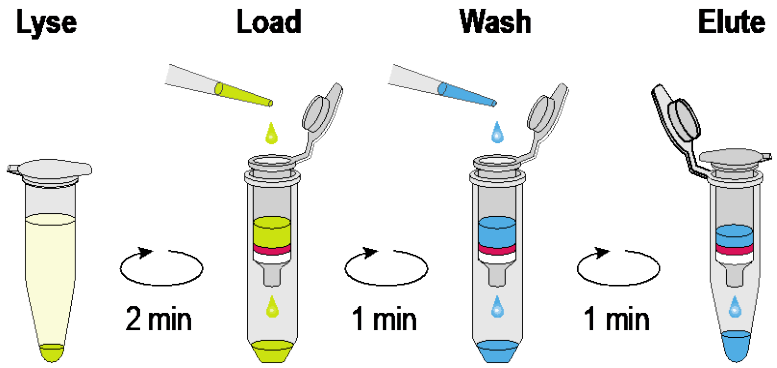
Additional materials and equipment required

- 96-100% Ethanol
- Pipettes, sterile nuclease-free tips
- 1.5 mL nuclease-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)
- Water bath or heating block (heated to 55°C).
- 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)

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 columns 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 spin columns and washed to remove protein, salt and other contaminants. Finally, purified nucleic acid is eluted from the column into a clean microcentrifuge tube 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™ DNA and RNA Purification Columns have a very high > 150 µg binding capacity and efficiently bind both DNA and RNA nucleic acids.

Experimental Workflow



Important Notes

BEFORE USE:

- Add 96-100% Ethanol to the **Wash Buffer WB-R2**:
  - **#NK055-50** (50 preps): add **45 mL** of Ethanol
  - **#NK055-250** (250 preps): add **170 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.
  - #NK055-250: reconstitute one tube at a time to prevent **Carrier RNA** degradation

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Lysis Buffer LB-V 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-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 spin column is significantly reduced. However, addition of Carrier RNA enhances the binding of low copy nucleic acids to the columns, 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.

Safety

Reagent	Risk Symbols	Risk and Safety Phrases
Lysis Buffer LB-V		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.

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**:
  - Add 5 µL of 1 µg/µL of **Carrier RNA** solution to each 300 µL of **Lysis Buffer LB-V** used.
  - Add 10 µL of 10 mg/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)

Step	Procedure
1	Sample prep: a) Purification from samples in Viral Transport Medium (VTM) ▪ Centrifuge VTM tubes containing specimen at maximum speed for 1 min ▪ Use 200 µL collected from the bottom of the tube. 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 2. 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 microcentrifuge tube. ▪ Seal the tube and incubate for 10 min at 55 °C ▪ Remove the swab tip and move directly to Step 5. ▪ **NOTE: Omit steps 2-4 below if using this sample source.** e) 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 2.
2	Transfer 200 µL of the sample or resuspension buffer from Step 1 to a new nuclease-free 1.5 mL microcentrifuge tube.

3	Add 300 µL of **Working Buffer LB-V** to the sample. Mix well by pipetting, or vortexing.
4	Incubate the samples for 10 min at 55°C to allow Proteinase K to digest viral proteins.
5	Add 300 µL of 96-100% Ethanol to the sample. Invert a few times to mix.
6	Transfer 800 µL of the solution onto 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 750 µL of **Wash Buffer WB-R1** to the column. Centrifuge at 12,000 x g for 1 min. Discard the flow-through.
8	Centrifuge at 12,000 x g for 1 min to dry out the membrane.
9	Add 750 µL of **Wash Buffer WB-R2** (*with Ethanol added*) to the column. Centrifuge at 12,000 x g for 1 min. Discard the flow-through.
10	Centrifuge at 12,000 x g for 1 min to dry out the membrane.
11	Transfer the **PuroSPIN™ MINI Spin Column** into a new nuclease-free 1.5 mL microcentrifuge tube.
12	Pipette 50 µL of **Elution Buffer EB** or **nuclease-free water** onto the center of the column. Centrifuge at 12,000 x g for 1 min to elute the RNA. Keep the flow-through that contains the product and discard the column.

Troubleshooting

Problem	Possible causes and solutions
Column clogging	**Starting material was not completely disrupted.** Perform more thorough homogenization. Use smaller amount of starting material. **Precipitates were not completely removed.** When using plasma samples, remove visible kryoprecipitates by centrifugation for 3,000g 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 column. **Ethanol was not added to Wash Buffer WB-R2** 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.** Perform more thorough homogenization. Use smaller amount of starting material. **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.** Use recommended 50 µL of Elution Buffer EB.
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 below -20°C.