User Guide

GenElute™ Viral RNA Miniprep Kit

RNV100

Product Description

The GenElute™ Viral RNA Miniprep Kit is designed for isolation of clean viral RNA using a silica-based micro spin column, eliminating the need for hazardous organic compounds such as phenol and chloroform. Isolation of viral RNA from saline, viral transport medium, saliva, cell suspension, and tissue culture medium can be performed using the same provided protocol.

In this method, viral particles are lysed in a buffer containing guanidine thiocyanate which aids in the denaturation of macromolecules and inactivation of RNases. Addition of ethanol to the lysate facilitates RNA adhesion to the silica membrane on the spin column. Membrane wash steps remove residual salts and contaminants, and the subsequent addition of Elution Buffer to the membrane mediates retrieval of clean RNA. This kit does not separate viral from cellular RNA and will co-purify host cellular RNA if present. Efficient purification of low concentrations of viral RNA is enhanced by the inclusion of poly(A) carrier RNA. Undesired DNA contamination can be removed with DNase I, either on-column while RNA is bound using the On-Column DNase I Digestion Set or after elution with Amplification Grade DNase I. Downstream compatible applications for purified RNA include cDNA synthesis, RT-qPCR, Next Generation Sequencing, and other standard RNA techniques.

Material and Equipment Needed

Kit Contents

	RNV100-70RXN 70 preps	**RNV100-350RXN** 350 preps
Lysis Solution	70 mL	5 x 70 mL
Poly(A) Carrier RNA	1 mg	5 mg
2-Mercaptoethanol (2-ME)	0.9 mL	2 × 2 mL
Wash Solution 1	40 mL	200 mL
Wash Solution 2 Concentrate	15 mL	75 mL
Elution Solution	10 mL	50 mL
GenElute Binding Columns	70 each	5 × 70 each
Collection Tubes, 2.0 mL capacity	3× 70 each	15 × 70 each

Not Supplied with Kit

- Ethanol
- 70% Ethanol solution
- RNase-free pipette tips (aerosol barrier recommended)
- Microcentrifuge capable of holding 2 mL tubes

OPTIONAL: On-Column DNase I Digestion Set

OPTIONAL: Amplification Grade DNase I

Precautions and Disclaimer

The GenElute Viral RNA Miniprep Kit is for laboratory use only, not for drug, household, or other uses.

Lysis Solution and Wash Solution 1 contain guanidine thiocyanate, which is harmful. Wear gloves, safety glasses, and suitable protective clothing when handling these solutions or any reagent provided with the kit. Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

RNases are ubiquitous and stable proteins, which are a concern for any researcher attempting to isolate RNA. The prepared Lysis Solution contains guanidine thiocyanate and 2-mercaptoethanol, both of which inactivate RNases. Conditions during column binding and the first wash remove RNases.

Care must be taken not to introduce RNase, especially during the final wash and elution. Use RNase-free pipette tips, preferably those having an aerosol barrier. Keep bottles and tubes closed when not adding or removing their contents.

Storage and Stability

Store the kit at 15-30 °C. If any reagent forms a precipitate, warm at 65 °C until the precipitate dissolves and allow to cool to 15-30 °C before use.

Standard Protocol

Preparation

1. Reconstitute and aliquot Poly(A) Carrier RNA

Briefly centrifuge Poly(A) Carrier RNA vial such that lyophilized material collects at the bottom. Carefully open the vial.

- For 70 prep kit (contains 1 mg carrier RNA) add 1 mL Lysis Buffer to bring carrier RNA concentration to 1 mg/mL.
- For 350 prep kit (contains 5 mg carrier RNA) add 1 mL Lysis Buffer, transfer to a larger RNase-free tube, and add an additional 4 ml Lysis Buffer to bring concentration to 1 mg/ml.

Either use resuspended material immediately or aliquot as desired and store at -80 °C. If aliquoting plan for using 10 µL of carrier RNA per miniprep.

NOTE: Actual carrier RNA quantity may be up to 1 mg more than amount on the label. This will not affect miniprep functionality or yield.

2. Add Ethanol to Solution 2 Concentrate

Dilute the Wash Solution 2 Concentrate with 60 mL (70 prep kit), or 300 mL (350 prep kit) of 200 proof ethanol. After each use, tightly cap the diluted Wash Solution 2 to prevent evaporation of the ethanol.

Procedure

All steps should be carried out at 15-30 °C. It is important to move swiftly through the procedure.

Unless otherwise noted, centrifuge samples at 13,000-16,000 RPM using a tabletop centrifuge. A vacuum manifold may be used for steps 4-7, although excess ethanol should still be removed via centrifugation at the end of step 7. Ensure all liquid has passed through before proceeding to the next step.

The standard protocol below uses 120 µL sample size. If RNA extraction from a smaller or larger sample size is desired, proportionally scale the addition of lysis solution and 70% ethanol.

Example
If sample size is 180 µL, then add 750 µL of prepared Lysis Solution followed by 750 µL of 70% ethanol

Lysate can be added to the spin column across several loads, as long as the flowthrough is discarded between each load. Briefly centrifuge lysate before loading on to column to ensure all contents are at the bottom of the tube and retrievable by pipetting.

1. Lysis Solution Preparation with Carrier RNA and 2-Mercaptoethanol

Prepare Lysis Solution: For each miniprep to be performed, add 10 µl of 1 mg/ml Poly(A) carrier RNA and 5 µl of 2-mercaptoethanol to 500 µL of Lysis solution.

Number of Minipreps	Lysis solution µL	1 mg/mL Poly(A) Carrier RNA µL	2-Mercaptoethanol µL
1	500	10	5
2	1000	20	10
4	2000	40	20
8	4000	80	40
n	500 x n	10 x n	5 x n

2. Sample Lysis

Place 120 µL of sample containing virus in either the provided collection tube or a 1.5 mL tube. Add 500 µL of Lysis Solution containing Carrier RNA and 2-mercaptoethanol to the tube. Tightly cap tube and mix by pulse-vortexing for 10 seconds. Incubate sample at room temperature for 3-5 minutes.

3. **Preparation For Binding**

   Add 500 µL of 70% ethanol solution to lysate. Mix by pulse-vortexing for 10 seconds and immediately move on to the next step.

4. **Column Binding**

   Place a binding column into a 2 mL collection tube. Pipette up to 700 µL of the lysate/ethanol mixture onto the column and centrifuge at maximum speed for 15 seconds. Liquid flowthrough can be discarded but the column and collection tube should be retained. Continue adding lysate/ethanol, centrifuging, and discarding liquid flowthrough until all material has been loaded and no flowthrough remains in the collection tube.

   OPTIONAL: Continue with the procedure described in the Optional On-Column DNase I Digestion.

5. **First Column Wash**

   Add 500 µL of Wash Solution 1 on to the column and centrifuge at maximum speed for 15 seconds. Discard liquid flowthrough.

6. **Second Column Wash**

   Ensure that Wash Solution 2 Concentrate has been diluted with ethanol as described in the Preparation Instructions. Pipette 500 µL of this ethanol containing Wash Solution 2 into the column and centrifuge at maximum speed for 15 seconds. Discard liquid flowthrough.

7. **Third Column Wash and Drying**

   Pipette a second 500 µL volume of Wash Solution 2 into the column and centrifuge at maximum speed for 15 seconds. Discard liquid flowthrough.

   Centrifuge the column for an additional 2 minutes without any liquid to remove any residual ethanol that could interfere with RNA recovery and detection.

8. **RNA Elution**

   Transfer the binding column to a fresh 2 mL collection tube. Pipette 50 µL of Elution Solution into the binding column and incubate at room temperature for 3-5 minutes. Centrifuge at maximum speed for 1 minute. Viral RNA is now present in eluate.

   NOTE: Using an elution volume less than 50 µL has not been observed to improve viral RNA concentration or yield.

Results

Analysis of RNA

Eluted viral RNA is ready for direct use in most downstream applications. If using same-day, keep RNA on ice or at 4-8 °C. For later use, store RNA at -70 °C. Specific targets can be detected using either a probe-based RT-qPCR or with SYBR® Green Dye.

While beneficial for purifying small amounts of viral RNA, Poly(A) Carrier RNA will appear in the eluate and interfere with spectrophotometric determination of RNA quantities. Carrier RNA can thus be excluded if purifying large amounts of viral RNA. Concentration of total RNA can be calculated by measuring absorbance at 260 nm with an extinction coefficient of 0.025 $(\mu g/mL)^{-1}$ cm^{-1}. A260/A280 ratio for pure RNA should be between 1.8 and 2.1. Note that the eluate will still likely contain any cellular RNA that may have been present in the original sample.

Treatment of eluate with DNase is recommended for applications such as cDNA synthesis. For highly sensitive applications, treatment of eluate with amplification-grade DNase is recommended.

Optional On-Column DNase I Digestion

To Be Performed During Standard Protocol

The majority of DNA is eliminated from RNA preparations by the purification procedures described in the Standard Protocol. Significantly lower levels of DNA contamination can be achieved by digesting with DNase I using the On-Column DNase I Digestion Set. For the most stringent removal of DNA, post-purification treatment with Amplification Grade DNase I is recommended.

1. Lyse sample and bind to column as described in steps 1-4 of the Standard Protocol.
2. Pipette 250 µL of Wash Solution 1 into the column and centrifuge at maximum speed for 15 seconds.
3. Mix 10 µL of DNase I with 70 µL of DNase Digest Buffer for each preparation. Mix by inversion or gentle pipetting. Do not vortex as DNase I is sensitive to physical denaturation.

   NOTE: The DNase I/Digest Buffer mixture may be prepared up to 2 hours in advance.
4. Add 80 µL of the DNase I/Digest Buffer mixture directly onto the filter in the Binding Column.
5. Incubate at room temperature for 15 minutes. Do not centrifuge until after adding additional Wash Solution 1 in the next step. Centrifuging at this step may result in significant loss of RNA.
6. Pipette 250 µL of Wash Solution 1 into the column and centrifuge at maximum speed for 15 seconds.
7. Continue through the remainder of the procedure beginning at step 6, the first wash with Wash Solution 2.

Troubleshooting

Problem	Cause	Solution
Binding column is clogged.	Sample background is viscous or complex.	Extend centrifugation time and increase speed if possible until all material has passed through. If problem persists reduce sample size and dilute to 120 µL.
Low yield or RNA degraded.	Sample contains small amounts of virus.	Increase sample size and scale Lysis Buffer and 70% ethanol accordingly. Additional Poly(A) carrier RNA can be purchased separately and added to lysis buffer but should not exceed 100 µg/mL final concentration.
	RNase contamination in eluate.	Ensure that all tips and plasticware are RNase-free. Perform work in a dedicated clean area, such as a laminar flow hood. Pretreat work area with RNaseZAP™ cleaning agent. Keep samples on ice after elution, or immediately freeze at -70 °C for future use.
	Omitted column wash with Wash Solution 1 after On-Column DNase I Digestion.	For future preparations, be sure to wash the column with 0.25 mL of Wash Solution 1 after DNase I digestion See Optional On-Column DNase I Digestion, step 7. Use of Wash 1 before Wash 2 is essential for full recovery of RNA.
	Complex sample such as serum or saliva.	Dilute sample 1:2 - 1:5. Complex "dirty" samples such as serum or saliva can contain a large number of biomolecules, along with proteases and/or inhibitors. Dilution can often allow for more efficient RNA stability and binding to the column.
Unacceptable level of DNA contamination in purified RNA.	Optional DNase I treatment was omitted.	Treat samples with the optional on-column DNase I digestion, see Optional On-Column DNase I Digestion. Alternatively, treat eluted RNA using Amplification Grade DNase I.
Downstream applications are inhibited.	Residual ethanol in eluate.	Residual ethanol from Wash Solution 2 can inhibit transcription enzymes. Take extra precaution to ensure that column does not come in contact with flowthrough when transferring to a new collection tube after the drying step, right before elution. If problem persists an additional 1-2 minutes of centrifugation can be added to the drying step.
	Residual salt in eluate.	Residual guanidine thiocyanate will also inhibit enzymes. Ensure that all washes are being performed properly and thoroughly.

Product Ordering

Order Online at SigmaAldrich.com

Description	Qty	Catalogue Number
GenElute™ Viral RNA Miniprep Kit	70 preps	RNV100-70KT
	350 preps	RNV100-350KT

Additional Products

Ethyl alcohol, Pure, 200 proof for molecular biology	multiple quantities*	E7023
On-Column DNase I Digestion Set	10 preps 70 preps	DNASE10 DNASE70
DNase I, Amplification Grade	1 kit	AMPD1
ReadyMix™ Quantitative RT-PCR	1 kit	QR0200
KiCqStart® One-Step Probe RT-qPCR, ready mix	500 reactions	KCQS07
KiCqStart® One-Step Probe RT-qPCR, ready mix with low Rox™ fluorescent dye	500 reactions	KCQS08
KiCqStart® One-Step Probe RT-qPCR, ready mix with Rox™ fluorescence dye	500 reactions	KCQS09
RNaseZAP™ Cleaning Agent	multiple quantities*	R2020
Poly(A), Polyadenylic acid	multiple quantities*	P9403

*multiple pack quantities/sizes available, specify when ordering

Notice

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GenElute™ Viral RNA Miniprep Kit Lab Checklist

All spins at 13,000-16,000 × g.

Release and Prepare RNA

For larger samples, lysis solution and 70% ethanol amounts can be scaled up proportionally.

1. Add 2-mercaptoethanol (10 µL/mL) and Poly(A) carrier RNA (20 µg/mL) to lysis solution.
2. Lyse 120 µL of virus-containing sample with 500 µL of supplemented lysis solution. Mix by vortexing.
3. Incubate 3-5 minutes at room temperature.
4. Add 500 µL of 70% ethanol to lysate. Mix by vortexing.

Bind RNA to Column

1. Transfer up to 700 µL lysate/ethanol mixture to binding column.
2. Spin ≥15 seconds and discard flow-through.
3. Repeat as needed until entire mixture has been loaded.

Wash to Remove Contaminants

Ethanol must be added to Wash 2 concentrate before first use.

1. Add 500 µL **Wash Solution 1** to column.
2. Spin ≥ 15 seconds and discard flow-through.
3. Add 500 µL **Wash Solution 2** to column.
4. Spin ≥ 15 seconds and discard flow-through.
5. Add 500 µL **Wash Solution 2** to column.
6. Spin ≥ 15 seconds and discard flow-through.
7. Spin 2 minutes to remove any residual ethanol.

Elute Purified RNA

1. Transfer column to new collection tube.
2. Add 50 µL elution solution to column.
3. Incubate 3-5 minutes at room temperature.
4. Spin 1 minute to elute RNA.