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GeneJET™ Viral DNA and RNA Purification Kit

USER GUIDE

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For Research Use Only. Not for use in diagnostic procedures.

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Contents

Product information

Product description

The GeneJET™ Viral DNA and RNA Purification Kit is designed for rapid and efficient purification of high quality viral nucleic acids from various human and animal liquid samples such as plasma, serum, whole blood, saliva, nasal and buccal swabs, urine (cells should be collected before purification), urogenital swabs and milk.

The kit utilizes a silica-based membrane technology in the form of a convenient spin column. Nucleic acids from lysed samples bind to the column membrane while impurities are effectively removed during subsequent washing and centrifugation steps. Finally, ready-to-use nucleic acids are eluted from the column.

The purified viral nucleic acids are free of proteins, nucleases, and other contaminants or inhibitors of downstream applications. Isolated DNA and RNA can be directly used in PCR, qPCR or other nucleic acid based assays. Host genomic DNA or RNA co-purified from liquid cell-containing samples typically does not interfere with viral nucleic acid yields due to high capacity of the spin column membrane (up to 50 µg).

To minimize irregularities in results, the product must be used with an appropriate internal control as well as positive and negative controls throughout the process of sample preparation, amplification and detection.

Contents and storage

Upon receipt, store each component as instructed. All components are stable until the listed expiration date. Wash Buffer 1 and Wash Buffer 2 are stable until the listed expiration date after addition of ethanol.

Component	Amount	Storage
Column Preparation Liquid (red cap)	2 × 1.4 mL	Room temperature (15–25°C)
Lysis Solution [1]	12 mL	
Wash Buffer 1 (concentrated) [1]	25 mL	
Wash Buffer 2 (concentrated)	11 mL	
Eluent (white cap)	3 × 1.25 mL	
Proteinase K (green cap)	2 × 1.3 mL	–20°C
Carrier RNA, dried (blue cap)	1 vial	–20°C in foil pouch
Spin Columns preassembled with Wash Tubes [2]	50 tubes	Room temperature
Wash Tubes (2-mL)	4 × 50 tubes	

[1] Contains guanidine hydrochloride (guanidinium chloride).
[2] Close the bag with Spin Columns tightly after each use.

2 Methods

Important guidelines

- Ensure the integrity of the kit components upon the delivery. Contact technical support or your local distributor in case of damage. Do not use damaged kit components.
- The Lysis Solution and Wash Buffer 1 contain irritants. Always wear gloves and follow standard safety precautions when handling these reagents. For more information refer to the Material Safety Data Sheets.
- All sample material and waste should be regarded as potentially infectious. Wear the proper protection when handling samples and waste. Avoid any skin or eye contact.
- Work under laminar air flow conditions if possible until samples are lysed. Disinfect all work surfaces thoroughly after the procedure. Follow proper waste disposal guidelines as recommended by local authorities.
- The following steps should be taken in order to avoid cross-contamination:
  - Always change pipette tips between liquid transfers (aerosol-barrier pipette tips recommended)
  - Open only one tube at a time
  - Use disposable gloves and discard if contaminated
- Always use RNase-free equipment.
- Use only a freshly prepared mixture of Carrier RNA and Lysis Solution when beginning a new extraction procedure.
- Before beginning the procedure, a new Spin Column must be prepared by adding 50 µL of Column Preparation Liquid into the center of the column membrane. Do not centrifuge the column after addition of Column Preparation Liquid.

About internal controls

The presence of an internal control throughout the extraction and purification procedure may be necessary for certain assays. Refer to the user manual provided with your downstream detection assay for information on how to use an internal control.

Guidelines for handling samples

- If possible, use only fresh sample material.
- Store **plasma**, **serum**, and **whole blood** samples at 2-8°C for up to 24 hours, or at –20°C or –70°C for long term storage.
- Store **urogenital swabs** at 2-8°C for up to 48 hours. For longer term storage, collect cells by centrifugation and store at –20°C or –70°C.
- Store **nasal and buccal swabs** at 2-8°C for up to 48 hours.
- Store **urine** samples at 2-8°C for up to 12 hours (with 0.5 M EDTA added to 50 mM final concentration), or at –20°C or –70°C for long term storage (collect cells by centrifugation). For viral RNA purification, collect cells by centrifugation immediately after sample collection.
- Do not freeze/thaw samples more than once.
- Equilibrate samples to room temperature (20±5°C) before use. Remove any precipitates from plasma/serum samples by centrifugation for 5 minutes at 3,000 × *g*.
- Use EDTA or citrate treated plasma samples.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Table 1 Materials required for lentiviral vector production

Item	Source
Pipettes and sterile, nuclease-free pipette tips with aerosol barrier	MLS
Laboratory mixer (Vortex mixer or equivalent)	MLS
Microcentrifuge	MLS
Thermomixer	MLS
Disposable gloves	MLS
Measuring cylinder	MLS
Nuclease-free microcentrifuge tubes of an appropriate size for preparing mixtures of Carrier RNA and Lysis Solution	MLS
Microcentrifuge tubes for sample lysis and elution	MLS
RNase-free tubes should be used for RNA elution	MLS
Ethanol (96-100%)	MLS

Before each use of the kit

General Guidelines

- Read the user manual; make sure all the directions are followed as indicated.
- Make sure all working solutions and samples have been prepared according to recommendations.
- Ensure all necessary equipment and additional materials are available before beginning the procedure.
- Organize proper handling of potentially infectious waste before beginning the procedure.
- Follow recommended safety instructions, as you will be working with potentially infectious material.
- Attention and care must be taken during the entire process.
- All centrifugation steps must be performed at room temperature.

Guidelines for preparing reagents and buffers

- Ensure all working solutions are prepared according to the recommendations in the protocol.
- After preparing each solution, mark the bottle to indicate that this step has been completed.
- Check all solutions in the kit for any salt precipitation before each use. Re-dissolve any precipitate by warming the solution to 37°C, and then equilibrate to room temperature (20±5°C).
- For swabs or collected cells, reconstitute cells to recommended sample volume using PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH2PO_4, pH 7.4)
- It is the user's responsibility to use appropriate controls during the procedure.

Prepare Wash Buffers

Add the indicated volume of ethanol (96-100%) to the concentrated Wash Buffer 1 and concentrated Wash Buffer 2 prior to first use.

Component	Wash Buffer 1	Wash Buffer 2
Concentrated wash buffer	25 mL	11 mL
Ethanol (96-100%)	15 mL	44 mL
Total volume	40 mL	55 mL

About Carrier RNA

Carrier RNA is provided in a dried state packed in a moisture-impermeable aluminum bag.

Use of Carrier RNA is important for efficient recovery of viral nucleic acids for two reasons.

1. Carrier RNA facilitates binding of viral nucleic acids to the silica membrane, especially when there are only a small number of viral nucleic acid molecules in the sample.
2. In the rare event when there are a small number of active RNase molecules, large amounts of Carrier RNA reduce the probability of viral RNA being degraded under chaotropic conditions.

If Carrier RNA is not added to the Lysis Solution, reduced viral nucleic acid yields may result.

Prepare Carrier RNA

1. Reconstitute the dried Carrier RNA by adding 300 µL of Eluent to the tube.
2. Incubate the freshly reconstituted Carrier RNA to for 5 minutes at room temperature, then mix thoroughly and briefly centrifuge the vial.
3. Use immediately or store at –20°C.

   Note: Do not freeze-thaw the reconstituted Carrier RNA more than 10 times.

4. (*Optional*) If only few samples are processed at a time, Divide the Carrier RNA solution into 50 µL aliquots (using nuclease-free tubes) and store at –20°C or –70°C.

How to calculate required amount of Carrier RNA

When starting a new procedure, always use a freshly prepared mixture of Carrier RNA and Lysis Solution. To calculate the required volume (µL) of Carrier RNA use the following formulas:

- N × 0.22 mL = Y mL
- Y mL × 25.0 µL/mL = Z µL

Where N is the number of samples to be processed, Y is the calculated volume (mL) of Lysis Solution, and Z is the volume (µL) of Carrier RNA to add to Y mL of Lysis Solution.

Add the required quantity of Carrier RNA to the Lysis Solution, then mix by pulse-vortexing or pipetting.

Table 2 Pre-calculated volumes of Carrier RNA and Lysis Solution required to process multiple samples

No. samples	Lysis Solution	Carrier RNA	No. samples	Lysis Solution	Carrier RNA
1	0.22 mL	5.5 µL	13	2.86 mL	71.5 µL
2	0.44 mL	11 µL	14	3.08 mL	77 µL
3	0.66 mL	16.5 µL	15	3.30 mL	82.5 µL
4	0.88 mL	22 µL	16	3.52 mL	88 µL
5	1.10 mL	27.5 µL	17	3.74 mL	93.5 µL
6	1.32 mL	33 µL	18	3.96 mL	99 µL
7	1.54 mL	38.5 µL	19	4.18 mL	104.5 µL
8	1.76 mL	44 µL	20	4.40 mL	110 µL
9	1.98 mL	49.5 µL	21	4.62 mL	115.5 µL
10	2.20 mL	55 µL	22	4.84 mL	121 µL
11	2.42 mL	60.5 µL	23	5.06 mL	126.5 µL
12	2.64 mL	66 µL	24	5.28 mL	132 µL

Purification of viral nucleic acid (main protocol)

This protocol is for viral DNA and RNA purification from 200 µL of EDTA- or citrate- treated plasma, serum, blood or milk samples. For other sample types see “Purification of viral nucleic acid (other sample types)” on page 12. Following procedure provides instruction for processing one sample. When using larger than 200 µL (up to 400 µL) sample volumes see “Purification of viral nucleic acid (large sample volume)” on page 13.

Prepare Spin Column

Before starting the procedure, each new Spin Column must be prepared by treating it with Column Preparation Liquid. Column treatment maximizes binding of the nucleic acids to the membrane, resulting in more consistent yields.

IMPORTANT! Close the bag with Spin Columns tightly after each use

1. Add 50 µL of Column Preparation Liquid to the center of Spin Column membrane, so that the membrane is entirely moistened.
2. Do not centrifuge the prepared column. The prepared column should be stored at room temperature until it is used for sample processing.

Lyse sample

1. Add 200 µL of sample to an empty 1.5-mL lysis tube.
2. Add 200 µL of Lysis Solution (supplemented with Carrier RNA), and 50 µL of Proteinase K, mix thoroughly by vortexing or pipetting.

   Note: Prepare Lysis Solution with Carrier RNA prior to use (see “How to calculate required amount of Carrier RNA” on page 9). Use the appropriate internal control as required in a downstream assay user’s manual. Do not add the internal control directly to plasma samples. Do not add Proteinase K directly to Lysis Solution.
3. Incubate the sample for 15 minutes at 56°C in a thermomixer. Leave thermomixer turned on for Eluent preheating during later steps of the procedure.
4. Centrifuge for 3–5 seconds at full speed to collect any sample solution from the inside of the lid.

Bind sample

1. Add 300 µL of ethanol (96-100%) and mix by pipetting or vortexing.
2. Incubate the sample at room temperature for 3 minutes.
3. Centrifuge for 3–5 seconds at full speed to collect drops from the inside of the lid.

Wash sample

1. Transfer the lysate to the prepared Spin Column (see "Prepare Spin Column" on page 10) preassembled within the wash tube.
2. Centrifuge the column for 1 minute at 6,000 × *g*.
3. Discard the Wash Tube containing flow-through.
4. Place the Spin Column into a new 2-mL Wash Tube.
5. Add 700 µL of Wash Buffer 1 supplemented with ethanol (see "Prepare Wash Buffers" on page 8) to the Spin Column.
6. Centrifuge the column for 1 minute at 6,000 × *g*.
7. Discard the Wash Tube containing flow-through.
8. Place the Spin Column into a new 2-mL Wash Tube.
9. Add 500 µL of Wash Buffer 3 supplemented with ethanol (see "Prepare Wash Buffers" on page 8) to the Spin Column.
10. Centrifuge the column for 1 minute at 6,000 × *g*.
11. Discard the Wash Tube containing flow-through.
12. Place the Spin Column into a new 2-mL Wash Tube.
13. Centrifuge the column for 3 minutes at 16,000 × *g*.
14. Discard the Wash Tube containing flow-through.

Elute sample

1. Place the Spin Column into a new 1.5 mL elution tube.
2. Add 50 µL of Eluent preheated to 56°C to the center of Spin Column membrane.

   Note: To concentrate eluted nucleic acids, use a lower volume of Eluent (30–40 µL). qPCR and RT-qPCR inhibition might occur, if lower than 50 µL volume of Eluent is used for elution. Larger elution volumes (up to 100 µL) can also be used but may result in dilution of the viral nucleic acid sample.
3. Incubate for 3 minutes at room temperature.
4. Centrifuge the column for 1 minute at 13,000 × *g*.
5. Discard the Spin Column.

6. The elution tube contains pure viral nucleic acids. Use the purified nucleic acids immediately or store at –20°C or –70°C .

Note: For further use in downstream qPCR applications, use 1–10 µL of viral DNA per 25 µL reaction volume. For reverse transcription (RT), use 1–10 µL of viral RNA per 20 µL cDNA synthesis reaction volume.

Purification of viral nucleic acid (other sample types)

These protocols are for viral DNA and RNA purification from nasal and buccal swabs, urine, or saliva samples.

Nucleic acid purification from nasal and buccal swabs

1. Scrape the swab 5–6 times against the inside cheek or nose to collect a sample.
2. Swirl the swab for 2–3 min in 200 µL of 1X PBS or TE buffer.
3. Proceed with "Purification of viral nucleic acid (main protocol)" on page 10.

Nucleic acid purification from urine

1. Add 0.5 mL of 0.5 M EDTA to 4.5 mL of urine (final concentration 50 mM).

Note: Urine samples may contain insoluble salt precipitates that can reduce nucleic acid yields, thus urine samples larger than 4.5 mL are not recommended for purification.

2. Centrifuge 10 minutes at 800 × *g* (~3,000 rpm).
3. Discard the supernatant.
4. Resuspend the pellet in 200 µL of 1X PBS.
5. Proceed with "Purification of viral nucleic acid (main protocol)" on page 10.

Nucleic acid purification from saliva

1. Centrifuge the saliva sample for 5 minutes at 3,000 × *g* to collect cells.
2. Resuspend cells in 200 µL of 1X PBS or TE buffer.
3. Proceed with "Purification of viral nucleic acid (main protocol)" on page 10.

Purification of viral nucleic acid (large sample volume)

This protocol is for viral DNA and RNA purification for volumes up to 400 µL. Lysis Solution and ethanol are the only components that have to be scaled up.

Prepare Spin Column

Before starting the procedure, each new Spin Column must be prepared by treating it with Column Preparation Liquid. Column treatment maximizes binding of the nucleic acids to the membrane, resulting in more consistent yields.

IMPORTANT! Close the bag with Spin Columns tightly after each use

1. Add 50 µL of Column Preparation Liquid to the center of Spin Column membrane, so that the membrane is entirely moistened.
2. Do not centrifuge the prepared column. The prepared column should be stored at room temperature until it is used for sample processing.

Lyse sample

1. Add sample to an empty 2.0-mL lysis tube.
2. Add an equal volume (1:1) of Lysis Solution (supplemented with Carrier RNA), and 50 µL of Proteinase K to the sample. Mix thoroughly by vortexing or pipetting.

   Note: Prepare Lysis Solution with Carrier RNA prior to use (see "How to calculate required amount of Carrier RNA" on page 9). Use the appropriate internal control as required in a downstream assay user's manual. Do not add the internal control directly to plasma samples. Do not add Proteinase K directly to Lysis Solution.
3. Incubate the sample for 15 minutes at 56°C in a thermomixer. Leave thermomixer turned on for Eluent preheating during later steps of the procedure.
4. Centrifuge for 3–5 seconds at full speed to collect any sample solution from the inside of the lid.

Bind sample

1. Add 150 µL of ethanol (96-100%) for every 100 µL of initial sample volume. Mix by pipetting or vortexing.
2. Incubate the sample at room temperature for 3 minutes.
3. Centrifuge for 3–5 seconds at full speed to collect drops from the inside of the lid.

Wash sample

1. Transfer the lysate to the prepared Spin Column (see "Prepare Spin Column" on page 10) preassembled within the wash tube.

   Note: Do not load more than 700 µL of lysate on the column (For larger volumes, load the remaining volume of the lysate to the same column and centrifuge for a second time).

2. Centrifuge the column for 1 minute at 6,000 × *g*.
3. Discard the Wash Tube containing flow-through.
4. Place the Spin Column into a new 2-mL Wash Tube.
5. Add 700 µL of Wash Buffer 1 supplemented with ethanol (see "Prepare Wash Buffers" on page 8) to the Spin Column.
6. Centrifuge the column for 1 minute at 6,000 × *g*.
7. Discard the Wash Tube containing flow-through.
8. Place the Spin Column into a new 2-mL Wash Tube.
9. Add 500 µL of Wash Buffer 3 supplemented with ethanol (see "Prepare Wash Buffers" on page 8) to the Spin Column.
10. Centrifuge the column for 1 minute at 6,000 × *g*.
11. Discard the Wash Tube containing flow-through.
12. Place the Spin Column into a new 2-mL Wash Tube.
13. Centrifuge the column for 3 minutes at 16,000 × *g*.
14. Discard the Wash Tube containing flow-through.

Elute sample

1. Place the Spin Column into a new 1.5 mL elution tube.
2. Add 50 µL of Eluent preheated to 56°C to the center of Spin Column membrane.

   Note: To concentrate eluted nucleic acids, use a lower volume of Eluent (30–40 µL). qPCR and RT-qPCR inhibition might occur, if lower than 50 µL volume of Eluent is used for elution. Larger elution volumes (up to 100 µL) can also be used but may result in dilution of the viral nucleic acid sample.

3. Incubate for 3 minutes at room temperature.
4. Centrifuge the column for 1 minute at 13,000 × *g*.

5. Discard the Spin Column.

6. The elution tube contains pure viral nucleic acids. Use the purified nucleic acids immediately or store at –20°C or –70°C .

Note: For further use in downstream qPCR applications, use 1–10 µL of viral DNA per 25 µL reaction volume. For reverse transcription (RT), use 1–10 µL of viral RNA per 20 µL cDNA synthesis reaction volume.

A Troubleshooting

Observation	Possible cause	Recommended action
Column clogging	Starting material was not completely disrupted.	Reduce the amount of starting material and increase disruption time.
	Precipitates were not removed.	When using plasma samples, remove visible kryoprecipitates by centrifugation for 5 minutes at 3,000 × g.
Degraded RNA	RNase contamination.	To avoid RNase contamination, wear gloves during all procedures and change gloves frequently. Use sterile, disposable, RNase free pipette tips. Remove RNase contamination from non-disposable items and work surfaces.
	Poor quality of samples.	Always use fresh samples or samples handled as recommended in "Guidelines for handling samples" on page 7. For lysis, process the sample quickly to avoid degradation.
Low nucleic acid yield	Improper spin column preparation.	Make sure the Spin Column has been prepared properly by adding Column Preparation Liquid **as described on page 5**.
	Ethanol was not added to the lysate.	Make sure that ethanol was added to the lysate before applying the sample to the purification column.
	Ethanol was not added to Wash Buffers 1 and 2.	Make sure that ethanol was added to Wash Buffers 1 and 2 before the first use.
	Low percentage ethanol used.	Use only 96-100% ethanol. Do not use denatured or 95% ethanol.
	Carrier RNA was not added to the lysate.	Reconstitute carrier RNA in Eluent and mix with Lysis Solution **as described on page 5**.
	Degraded carrier RNA.	Do not freeze-thaw the reconstituted Carrier RNA more than 10 times. Store at –20°C to –70°C.
	Viral nucleic acid eluate too dilute.	Use recommended 30-50µL of Eluent.
	Improper elution conditions.	Apply preheated Eluent into the center of Spin Column membrane **as described on page 5**.