

User Manual

GenElute™-E Viral RNA/DNA Kits

For purification of viral RNA/DNA
from nasopharyngeal and genital swabs, and stool samples

EC810 EC848 EC896 EC888 EC996

Sigma-Aldrich®

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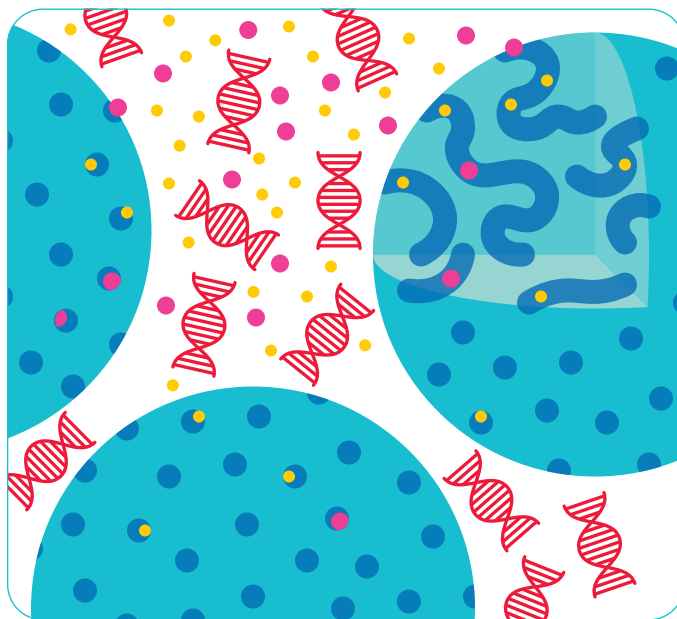
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Introduction

GenElute™-E Viral RNA/DNA Kit is a nucleic acid purification system that eliminates the need for binding and ethanol wash steps, yielding DNA and RNA preparations with robust results. GenElute™-E DNA and RNA purification kits employ a **negative chromatography** method dependent on size exclusion to separate large DNA and RNA nucleic acid molecules from smaller protein, lipid, and ionic components in cell, tissue, blood, and other samples.



Using negative chromatography, Single Spin columns and plates efficiently absorb and retain sample contaminants while allowing nucleic acids to flow through, reducing the number of steps and plastic materials required for purification. The key is the novel lysis that allows negative chromatography to be used for high quality nucleic acid purification.

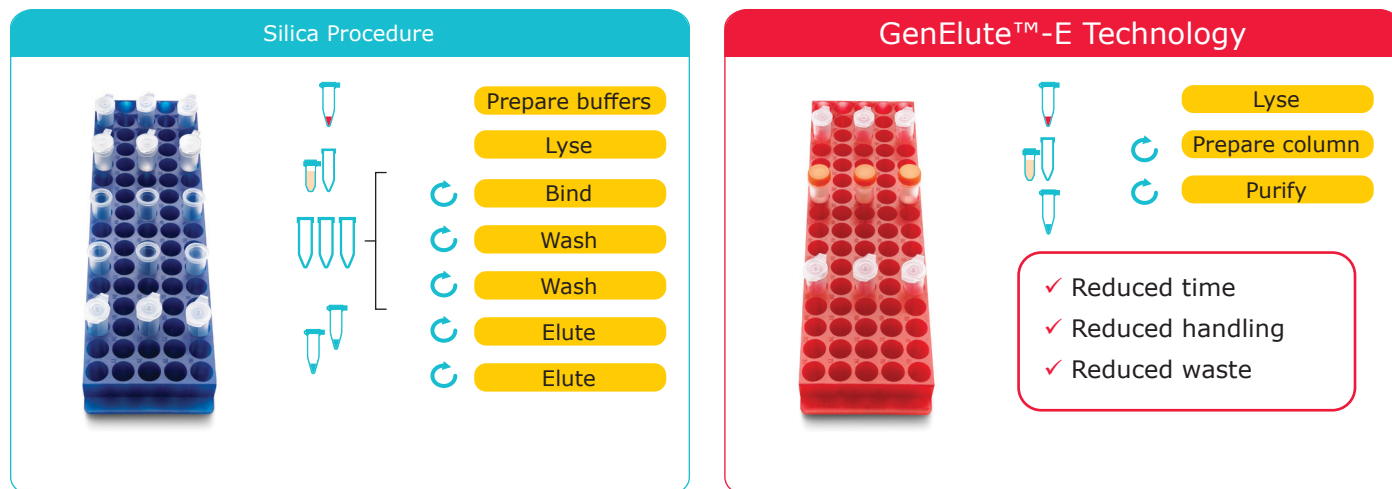
Three key advantages over silica:

- Simplified workflow
- Superior performance
- Waste reduction

For research use only.

A Simplified Workflow

Purification in one spin, eliminating all wash steps and reducing tube handling for more efficient, safer sample processing.



Reduced Waste for a Better Environment

With fewer plastic tubes and hazardous liquids, GenElute™-E DNA and RNA purification kits provide an eco-friendly alternative to silica-based purification.

GenElute™-E purification kits greatly reduce the amounts of plastic-based components packaged with each kit and consumed while executing protocols in the lab. All tedious binding and washing steps associated with silica-based procedures are omitted. Plastic waste is reduced by 55% compared to a common silica kits, resulting in disposal cost savings and reduced environmental impact.

GenElute™-E Single Spin nucleic acid purification kits provide easier workflows for DNA and RNA isolation, better nucleic acid quality, and reduced plastic and hazardous waste disposal compared to silica bind-wash-elute spin prep kits.

GenElute™-E Single Spin Purification supports:

- Significantly reduced plastic waste
- No hazardous bind and wash steps
- Responsible and sustainable nucleic acid purification
- Disposal cost savings



Specifications

Sample Input	Nasopharyngeal or genital swabs, or 10 – 20 mg stool
Sample Type	• dry nasopharyngeal swabs • nasopharyngeal swabs stabilized in transport medium* • genital swabs • stool samples
Required time after lysis	2 minutes
Purified Nucleic Acid	DNA > 500 bp
Elution Volume	70-90 µl
The purified RNA/DNA is ready for immediate use in downstream applications:	PCR and qPCR Sequencing reactions

* See Compatible Transport Media table.

Compatible Transport Media

Swabs can be collected with numerous swab types, both dry and with transport media. The following list of transport media has been successfully tested and are compatible.

Manufacturer	Name	Type
Copan	eSwabs™	non-chaotropic
Copan	UTM	non-chaotropic
Heinz Herenz Germany	LMS-SWAB	non-chaotropic
Hologic®	Specimen Lysis Tube	non-chaotropic
BioEcho Life Science GmbH	EchoSAFE Viral Transport Medium	chaotropic
Prestige Diagnostics	Single-Use Specimen Container	chaotropic
CoWin Biosciences	Viral Sample Preservation Solution	chaotropic

Manufacturer	Name	Type
Procomcure Biotech	PhoenixProtect RNA/DNA Conservation Solution	chaotropic
Roche	Cobas® PCR Media	chaotropic
In-house production	PBS or Tris Buffer	Resuspension of dry swabs

Storage and Stability

Kit Storage

GenElute™-E Single Spin Viral RNA/DNA Kits feature a shelf-life of 12 months from date of manufacture when stored at 2-8 °C.

Sample Storage and Variability

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Nucleic acid degrades over time, potentially leading to reduced fragment length and overall yield. Be aware that the yield to be expected is donor-dependent. To ensure optimal performance of the purified nucleic acids, proceed immediately with PCR/RT-PCR setup or any other downstream application. If this is not possible, store RNA at -70 °C and DNA at -20 °C and prevent freeze-thaw cycles.

Intended Use

For the purification of viral RNA and DNA from dry nasopharyngeal swabs or those stabilized in transport medium. Additionally, this product can be used with genital swabs as well as stool samples.

Typical Results

PCR specifications of heat-inactivated virus titer from swab samples

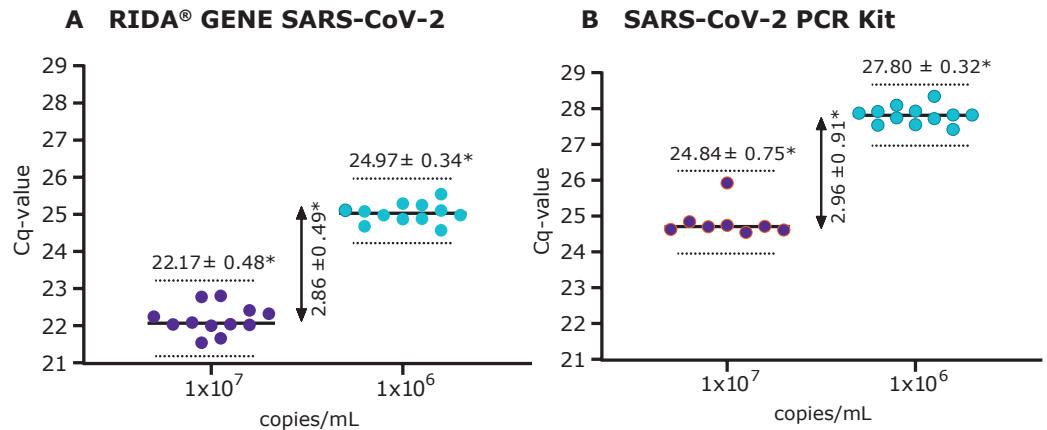
Linear Range	1×10^3 to 1×10^6 copies/ml
The correlation coefficient (R^2)	0.9995
LoD	13 copies/5 μ l PCR reaction

Assessment of linearity and limit of detection (LoD) using a PCR assay by RIDA® GENE SARS-CoV-2 (R-Biopharm) with non-chaotropic and chaotropic transport media spiked with heat-inactivated SARS-CoV-2 in a dilution series of 11 different virus titers (5×10^1 to 1×10^6 copies/ml).

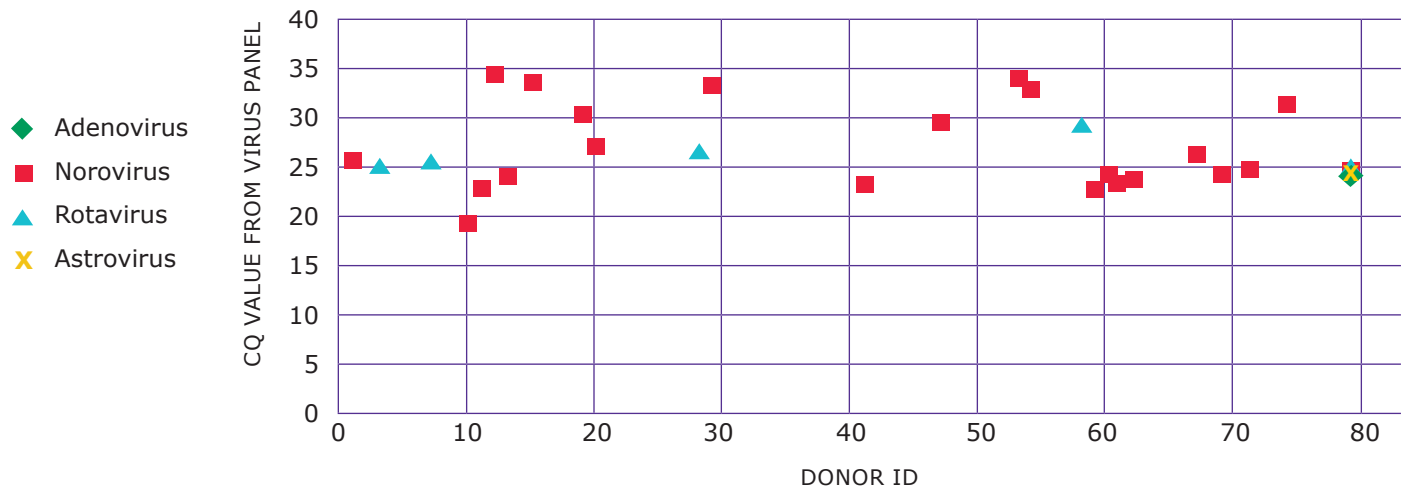
Swab sample Cq value differentiation between copy numbers

Successful participation in an interlaboratory test by INSTAND e.V. Scatter plot analysis reveals a significant ($p < 0.0001$) differentiation between reference sample 1 (1×10^7 copies/mL) and sample 2 (1×10^6 copies/mL) in a combination of the GenElute™-E Viral RNA/DNA Kit with either the (A) RIDA®GENE SARS-CoV-2

(R-Biopharm) or the (B) SARS-CoV-2 PCR Kit (Anchor Diagnostics) for E gene. A dilution factor of 7.3 and 7.8 was shown for the R-Biopharm (A) and Anchor Diagnostics (B) assays, respectively, which is within the calculated range across 234 labs that participated in the inter-laboratory test. 95% Confidence Interval (CI) is shown for each of the reference samples.



CQ Values from Stool Samples



Clinical validation for human norovirus extraction was investigated on a total of 83 patient samples.

The GenElute™-E Viral RNA/DNA Kit was used in combination with the RIDA®GENE Viral Stool Panel I (R-Biopharm) which is a multiplex real-time RT-PCR for the direct, qualitative detection and differentiation of Norovirus, Rotavirus, Adenovirus, and Astrovirus in human stool samples.

GenElute-E™ Viral RNA/DNA Swab Kit

Materials and Equipment Needed

Kit Contents

- SmartLyse™ Viral Buffer
- GenElute-E™ Spin Columns

Not Supplied in Kit

- Microcentrifuge with rotor for 1.5 mL and 2 mL reaction tubes.

Important: Switch centrifuge to relative centrifugal force, rcf (x g); if this is not possible please use formula to calculate the conversion of rotation per minute (rpm) into rcf. Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm matching the g-force using the formula:

$$\text{rpm} = 1,000 \times \sqrt{(g / (1.12 \times r))},$$

where r = radius of rotor in mm and g is the required g-force.

- Vortex device.
- Pipets for 10 µL and 200 µL volumes, corresponding pipette tips.
- One reaction tube (1.5 mL) per sample for sample preparation.
- One reaction tube (2 mL) per sample for column preparation.
- One reaction tube (1.5 mL) per sample for collection of the purified viral nucleic acids.

Optional: ICs that are added before the purification step should be >500 nucleotides in length.

Optional: 50mM Tris buffer or PBS of pH 7.2-8.5



Standard Protocol

Preparation before starting

Set the microcentrifuge to 1,000 x g.

Column Preparation

1. Vortex the GenElute™-E Spin Column briefly and place into a 2 mL reaction tube.
2. Let stand for 15 minutes.
3. Loosen the screw cap of the spin column and snap off bottom closure of the column. The screw cap must stay loosened half a turn to avoid generation of a vacuum. Place the spin column back into the 2 mL reaction tube.
4. Centrifuge for 1 minute at 1,000 x g. Discard the 2 mL reaction tube containing the column buffer.
5. Place the prepared GenElute™-E Spin Column into a new 1.5 mL reaction tube for collection of the purified viral nucleic acids and place back into the rack.

Lysis and Recovery of Viral Nucleic Acid

6. Add 50 µL of SmartLyse™ Viral Buffer to 1.5 mL reaction tube for sample preparation.

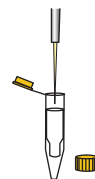
Optional: Add 1-20 µL of IC provided by user. ICs that are added before the purification step should be >500 nucleotides in length.

7. Preparing sample:
 - a. Swabs in transport media (e.g., Copan UTM, eSwab medium): Transfer 50 µL of sample to the 1.5 mL reaction tube containing SmartLyse™ Viral Buffer.

NOTE: With Amies agar swabs, avoid the carry-over of agar particles.
 - b. Dry Swabs: Dilute the viral particles by rinsing the swab in 300-700 µL PBS of pH 7.2-8.5. Transfer 50 µL of sample to the 1.5 mL tube containing SmartLyse™ Viral Buffer.
 - c. Stool samples: In a 2.0 mL tube add 10-20 mg stool sample and keep on ice. Resuspend in 600 µL 50 mM Tris buffer or PBS of pH 7.2-8.5. Transfer 50 µL of solution to the 1.5 mL tube containing SmartLyse™ Viral Buffer. Mix both by pipetting up and down several times.

8. Transfer 90 µL of sample solution to the prepared GenElute™-E Spin Column as illustrated:

Open cap and pipet the sample slowly (5 seconds) onto the center of the resin bed of the prepared spin column. Close screw cap and loosen again half a turn.



Important: Do not re-close the screw cap of the spin column completely.



Note: PCR inhibition observed with your PCR system can be eliminated by loading a reduced sample mixture volume to the column (75 µL or 50 µL) instead of 90 µL.

9. Centrifuge for 1 minute at 1,000 x g. The viral nucleic acid flows through the column into the 1.5 mL storage tube. Discard the spin column.

The collected viral nucleic acids can be used within two hours or stored at -20 °C (DNA) or -70 °C (RNA). Results are best when used within 3 days.

GenElute-E™ Viral RNA/DNA Swab Kits

48- or 96-well Plate

Materials and Equipment Needed

Kit Contents

- Lysis Plate: 96-well plate for lysis of samples.
- Purification Plate: 96-well plate containing the resin matrix for DNA purification.
- Elution Plate: 96-well plate for the collection of the purified DNA.
- Viral SmartLyse™ Buffer

Not Supplied in Kit

- Conditioning Plate: 48- or 96-deep well plate with minimum of 800 µL well volume for the collection of void volume during preparation of the Purification Plate. Reusable!
- Adhesive Foil for plate sealing during lysis.
- 96-well swing-bucket centrifuge with a minimum of 5 inches for the plate holder height

Important: Switch centrifuge to relative centrifugal force, rcf (x g); if this is not possible please use formula to calculate the conversion of rotations per minute (rpm) into rcf. Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm matching the g-force using the formula:

$$\text{rpm} = 1,000 \times \sqrt{(g / (1.12 \times r))},$$

where r = radius of rotor in mm
and g is the required g-force.

- Vortex device.
- Pipets for 10 µL, 200 µL, and 1,000 µL volumes, corresponding pipet tips.
- 8-channel pipets for 200 µL volume, corresponding pipet tips.
- Balance Plate(s) to be used in the centrifuge in case an odd number of plates is processed.

Optional: (IC) Internal Controls that are added before the purification step should be >500 nt in length

Optional: 50mM Tris or PBS of pH 7.2-8.5



Standard Protocol

Preparation before starting

Set the centrifuge to 1,000 x g.

Purification Plate Preparation

1. Carefully detach the lower and upper sealing foils from the Purification Plate.

Note: If the Purification Plate was not shipped or stored upright, resin may stick to the upper foil. In this case, shake plate until resin is removed from upper foil.

2. Plate preparation: Place the Purification Plate on top of a 96-deep well plate (Conditioning Plate, not supplied, minimum well volume of 800 µL) and centrifuge for 1 minute at 1,000 x g to collect the void buffer from the Purification Plate. Discard the flow-through (void volume) collected in the Conditioning Plate (Conditioning Plate can be emptied and reused).
3. Place conditioned Purification Plate on top of the Elution Plate for collection of purified viral nucleic acids.

Lysis and Recovery of Viral Nucleic Acids

4. Add 50 µL of Viral SmartLyse™ Buffer to applicable wells of Lysis Plate for sample preparation.

Optional: Add 1-20 µL of IC provided by user. Internal Controls that are added before the purification step should be >500 nt in length.

5. Preparing sample:

- Swabs in transport media (e. g., Copan UTM, eSwab medium): Transfer 50 µL of sample to the same wells of the Lysis Plate containing SmartLyse™ Viral Buffer.

NOTE: With Amies agar swabs, avoid the carry-over of agar particles.

- Dry Swabs: Dilute viral particles by rinsing swab in 300-700 µL of PBS pH 7.2-8.5. Transfer 50 µL of sample to the same wells of the Lysis Plate containing SmartLyse™ Viral Buffer.
- Stool samples: In a deep-well plate, add 10-20 mg stool sample and keep on ice. Re-suspend in 600 µL 50 mM Tris buffer or PBS of pH 7.2-8.5. Transfer 50 µL of solution to the same wells of the Lysis Plate containing SmartLyse™ Viral Buffer.

6. Transfer 90 µL of sample solution from Lysis Plate to Purification Plate.

Important loading instructions:

- Make sure that the 8-channel pipette during the loading step releases the lysate solution slowly and vertically, non-angular onto the middle of the resin surface.
- Do not punch pipette tip into the resin bed during loading of lysate.

Note: PCR inhibition observed with your PCR system can be eliminated by loading a reduced sample mixture volume to the column (75 µL or 50 µL) instead of 90 µL.

7. Centrifuge Purification Plate on top of the Elution Plate for 1 minute at 1,000 x g. The purified nucleic acids flow through the wells into the Elution Plate. Discard the Purification Plate.

The collected viral nucleic acids can be used within two hours or stored at -20 °C (DNA) or -70 °C (RNA). Results are best when used within 3 days.

Limitations

of GenElute™-E Viral RNA/DNA Spin Column and 48/96-well Plate Kits

- Strict compliance with the user manual is required for nucleic acid purification. Following good laboratory practices is crucial for the successful usage of the product. Appropriate handling of the reagents is essential to avoid contaminations or impurities.
- For swab samples, only materials specified for the detection of viral targets should be used. If a sample is not directly processed, store samples according to the manufacturer's instructions of the collection/transport tube before use.
- Samples stored in RNA/DNA Shield™ (Zymo Research) are not compatible with the nucleic acid extraction technology of the GenElute™-E Single Spin Viral RNA/DNA Kit.
- False-negative results may occur if a specimen is improperly collected, transported, stored, or handled. False-negative results may also occur if inadequate numbers of viral particles are present in the sample material.
- Internal Controls (IC) from respective downstream assays (not included in GenElute™-E Single Spin Viral RNA/DNA Kit and generally provided by the manufacturer of the downstream assay) must not be added directly to the sample and the IC should be > 500 nucleotides in length.
- The centrifuge rotor needs to be able to hold plate sandwiches of 5 cm of height.
- The proof of principle for the GenElute™-E Single Spin Viral RNA/DNA Kit was evaluated and confirmed using state-of-the-art PCR. Performance parameters are highly dependent on the used PCR assay and system. Also, there are only recommendations but no international standard for setting the Cq threshold, which could influence the results as well.
- Appropriate performance characteristics need to be established by the user, particularly in conjunction with any other downstream application. Any result shall be interpreted within the context of all relevant clinical and laboratory findings.
- Depending on the competitor kit used as a reference, the comparison will be different.

Troubleshooting

Problem	Probable Cause	Solution
Low yield	Individual samples have inherent variability. In addition, there is variability across different sample types. Optimization needs to be performed by the user to validate for their sample type.	Degraded nucleic acid fragments below < 60 bp are depleted during purification. Using fresh samples stored under appropriate conditions or stabilizing the samples will help to mitigate low sample yields.
	Using too much sample may result in overloading the capacity for separation.	Use the recommended sample load. Optimization needs to be performed by the user to validate for their sample type.
	Using too little of sample may result in low sample yields.	Use the recommended sample load. Optimization may need to be performed by the user if their sample type is low yielding.
	Centrifugation speeds and spin times have been optimized to acquire the fraction of sample containing the nucleic acid.	Verify that centrifugation was performed under the recommended conditions.
	If the preparation steps were performed incorrectly, then the separation resin will be packed incorrectly.	Verify that the preparation steps were performed according to the protocol.
Low sample volume	Loading too low of sample or too high of sample may result in sample volume loss. The loaded sample volume is required to be within the 70-90 µl as that volume is required to displace the buffer the resin is suspended in.	If the sample volume available to be loaded is below 90 µl, then bring the sample within the recommended range using lysis buffer. If the sample volume available to be loaded is above 110 µl (impacting results), then only load up to the recommended volume.
260/230 ratios appear to be "too low."	In some cases, the 260/230 ratios may be below the recommended range.	Downstream assays have not been shown to be compromised by lower 260/230 ratios using nucleic acid isolated using GenElute™-E kits.
White precipitate when mixed with the Viral SmartLyse™ buffer.	This indicates the presence of sodium dodecyl sulfate in the transport medium.	The white precipitate is completely removed by the purification matrix and a transparent eluate is obtained.
Occurrence of liquid in the Elution Plate after purification although no sample was applied.	After plate preparation, the matrix is not completely dried out and contains an insignificant amount of transport buffer that leads to further drainage of the matrix and some liquid continue to flow although no sample was loaded.	This is completely normal and no reason for concern.
Dried resin	In rare cases, the resin in the Columns or Purification Plates dry out during storage. This may be due to not storing them according to the recommended conditions.	Store GenElute™-E kits according to the recommended conditions.
Purification Plates with leaks	Although rare, improper sealing or too robust of turbulence can break the seal of the covering of the Purification Plates.	Ensure Purification Plates are efficiently sealed before introducing them to any agitation. If problem persists, reduce the turbulence by reducing the rpm.
Cross contamination from sample leaking when using cap puncher	If the cap puncher is used the sample can escape from the hole in the cap since the lysis buffer and sample mixture can create foam.	Do not use the cap puncher with this kit. Open screw cap of purification column, load directly on to the purification resin, and return cap to column. Remember to leave the cap loosened a quarter turn to prevent a vacuum from forming inside the column.

Product Ordering

GenElute™-E Viral Products

Description	Qty	Catalogue No.
GenElute™-E Viral RNA/DNA Swab Kit	50	EC810-50RXN
	250	EC810-250RXN
GenElute™-E Viral RNA/DNA Swab Kit 48-well Plate	2	EC848-2EA
	8	EC848-8EA
GenElute™-E Viral RNA/DNA Swab Kit 96-well Plate	2	EC896-2EA
	8	EC896-8EA
SmartLyse™ Viral Buffer	500 mL	EC888-500ML
GenElute™-E Conditioning Plate	2	EC996-2EA
	8	EC996-8EA

Additional GenElute™-E Products

Description	Qty	Catalogue No.
GenElute™-E Single Spin Blood DNA Kit	10	EC100-10RXN
	50	EC100-50RXN
	250	EC100-250RXN
GenElute™-E Single Spin Blood DNA High Yield Kit	10	EC200-10RXN
	50	EC200-50RXN
	250	EC200-250RXN
GenElute™-E Single Spin Tissue DNA Kit	10	EC300-10RXN
	50	EC300-50RXN
	250	EC300-250RXN
GenElute™-E Single Spin Cell Culture DNA Kit	10	EC400-10RXN
	50	EC400-50RXN
	250	EC400-250RXN
GenElute™-E Single Spin Plant DNA Kit	10	EC500-10RXN
	50	EC500-50RXN
	250	EC500-250RXN
GenElute™-E Single Spin DNA Cleanup Kit	10	EC600-10RXN
	50	EC600-50RXN
	250	EC600-250RXN
GenElute™-E Organic Solvent DNA Cleanup	10	EC700-10RXN
	50	EC700-50RXN
	250	EC700-250RXN
GenElute™-E Single Spin RNA Cleanup Kit	10	EC800-10RXN
	50	EC800-50RXN
	250	EC800-250RXN
GenElute™-E Tissue Stabilizer	100	EC111-100ML
	500	EC111-500ML

Description	Qty	Catalogue No.
GenElute™-E RNA Gel Loading Buffer	1	EC222-1EA
	5	EC222-5EA
GenElute™-E Single Spin Tissue DNA 96 Kit	2	EC396-2EA
	8	EC396-8EA
GenElute™-E Single Spin Plant DNA 96 Kit	2	EC596-2EA
	8	EC596-8EA
GenElute™-E Single Spin Blood DNA 96 Kit	2	EC196-2EA
	8	EC196-8EA
GenElute™-E Single Spin Cap Puncher	1	EC9999-1EA

Precautions and Disclaimer

This product is for research use only. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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Standard Warranty

The applicable warranty for the products listed in this publication may be found at [SigmaAldrich.com/terms](https://www.sigmaaldrich.com/terms).

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GenElute™-E Viral Spin Column Checklist



Prepare before starting

- ☐ Vortex GenElute™-E spin column and place in a 2 mL tube.
- ☐ Let stand for 15 minutes.
- ☐ Loosen screw cap of spin column.
- ☐ Snap off bottom closure. Place spin column back into 2 mL tube.
- ☐ Centrifuge 1 minute at 1,000 x g to collect column buffer.
- ☐ Place column in a 1.5 mL tube.



Lysis and Recovery of Viral Nucleic Acids

- ☐ Add 50 µL of Viral SmartLyse™ Viral Buffer to 1.5 mL tube for sample preparation.
Optional: Add 1-20 µL of internal control (IC) provided by user. ICs that are added before the purification step should be > 500 nucleotides in length.
- ☐ Preparing sample
 - Swabs in transport media: Transfer 50 µL of swab media to the 1.5 mL tube with SmartLyse™ Viral Buffer.
 - Dry Swabs: Dilute the viral particles by rinsing the swab in 300-700 µL PBS of pH 7.2-8.5. Transfer 50 µL of sample to the 1.5 mL tube with SmartLyse™ Viral Buffer.
 - Stool samples: In a 2.0 mL tube add 10-20 mg stool sample and keep on ice. Resuspend in 600 µL 50 mM Tris buffer or PBS of pH 7.2-8.5. Transfer 50 µL of solution to the 1.5 mL reaction tube containing SmartLyse™ Viral Buffer.
- ☐ Transfer 90 µL of sample solution to prepared columns.
- ☐ Centrifuge 1 minute at 1,000 x g to collect Viral RNA/DNA.
- ☐ Collected Viral RNA/DNA is ready to use



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20598313w-CL Rev 09/21 spin column

MERCK

GenElute™-E Viral Plate Checklist



Purification Plate Preparation

- ☐ Detach lower and upper sealing foils from Purification Plate.
- ☐ Place Purification Plate on top of a Conditioning Plate.
- ☐ Centrifuge 1 minute at 1,000 x g to collect void buffer.
- ☐ Place conditioned Purification Plate on top of Elution Plate.

Lysis and Recovery of Viral Nucleic Acids

- ☐ Add 50 µL of Viral SmartLyse™ Buffer to applicable wells of Lysis Plate for sample preparation.

Optional: Add 1-20 µL of internal control (IC) provided by user. Internal Controls that are added before the purification step should be >500 nt in length.

- ☐ Preparing sample:
 - Swabs in transport media: Transfer 50 µL of sample to the wells of the Lysis Plate containing SmartLyse™ Viral Buffer.
 - Dry Swabs: Dilute viral particles by rinsing swab in 300-700 µL of PBS pH 7.2-8.5. Transfer 50 µL of sample to the wells of the Lysis Plate containing SmartLyse™ Viral Buffer.
 - Stool samples: In a deep-well plate, add 10-20 mg stool sample and keep on ice. Resuspend in 600 µL 50 mM Tris buffer or PBS of pH 7.2-8.5. Transfer 50 µL of solution to the wells of the Lysis Plate containing SmartLyse™ Viral Buffer.
- ☐ Transfer 90 µL of sample solution from Lysis Plate to Purification Plate.
- ☐ Centrifuge 1 minute at 1,000 x g to collect viral nucleic acids into the Elution Plate.
- ☐ Collected Viral RNA/DNA is ready to use



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20598313w-CL Rev 09/21 plate

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