Quick Start

GenElute™-E Single Spin Viral RNA/DNA Swab Kit

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

EC810

Quick-Start Protocol (See Standard Protocol for detailed instructions.)

Column preparation

- 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 300-700ul 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.

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.

Storage and Stability

GenElute™-E Single Spin Viral RNA/DNA Swab Kit features shelf-life of 12 months from date of manufacture when stored at 2-8 ºC.

Materials and Equipment Needed

Kit Contents

- SmartLyse™ Viral Buffer
- 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:

  rpm = 1,000 x $\sqrt{(\ g/(1.12\ x\ r))}$,
  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: PBS of pH 7.2-8.5

Preparation before starting

Set the microcentrifuge to 1,000 x g.

Standard Protocol

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.

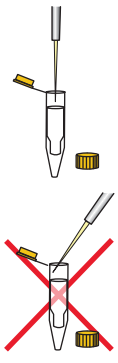
   b. Dry Swabs: Dilute the viral particles by rinsing the swab in 300-700ul PBS of pH 7.2-8.5. Transfer 50 µL of sample to the 1.5mL tube containing SmartLyse™ Viral Buffer.

   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.

Product Ordering

Purchase online at SigmaAldrich.com/products.

Description	Qty	Catalogue No.
GenElute™-E Single Spin 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

Precautions and Disclaimer

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

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