GET™ Viral Nucleic Acid

Genomic Efficient Technology for Viral Nucleic Acid purification from cell free biological samples (Cat. #786-1704, 786-1705)

INTRODUCTION

GET™ Viral Nucleic Acid belongs to our series of kits based on Genomic Efficient Technology (GET) for purification of nucleic acids from diverse sample.

GET™ is based on a highly efficient Genomic lysis buffer that liberates nucleic acid from cellular protein complexes, making nucleic acids free and available for purification in pure form. Free nucleic acids are immobilized, in the presence of high concentration of chaotropic agents, on silica solid phase membrane. Following the capture of nucleic acid on the silica membrane, a series of washing steps removes interfering impurities. In the final step, pure nucleic acid is eluted in concentrated form with elution buffer (Fig.1).

GET™ Viral Nucleic Acid is designed to extract and purify viral DNA from different cell-free biological samples such as serum, plasma, cell culture supernatant, nasal and throat swabs. The kit is designed to obtain highly pure viral RNA or DNA. The quality of the viral DNA or RNA obtained after purification is intact.

Furthermore, the kit is optimized to obtain high quality viral nucleic acid with minimum to no loss of nucleic acid. The purified nucleic acid fraction obtained is concentrated and is suitable for PCR, RT-PCR, qPCR and qRT-PCR studies. These PCR studies are used for virus detection, virus load, and virus genotyping.

GET™ Viral Nucleic Acid is available in 50 and 100 prep sizes with the maximum biological sample volume of 200 µl per prep.

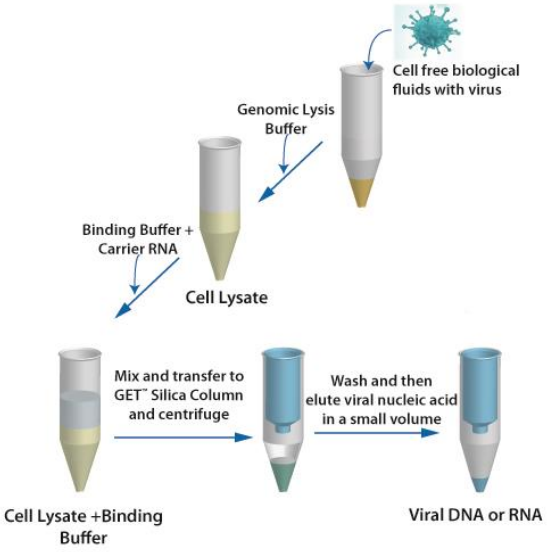


Fig:1

ITEMS SUPPLIED

Description	Cat. # 786-1704 50 preps	Cat. # 786-1705 100 preps
Genomic Lysis Buffer	30 ml	2 x 30 ml
GET Binding Buffer	50	2 x 50 ml
Poly A [Carrier RNA]	1 vial	2 vials
Longlife™ Proteinase K	0.5 ml	2 x 0.5 ml
GET Wash I	30 ml	2 x 30 ml
GET Wash II	20 ml	2x 20 ml
GET Elution Buffer	10 ml	10 ml
GET Silica Columns	50	100

STORAGE CONDITIONS

The kit is supplied at ambient temperature. Upon receipt store the kit components as indicated on labels. If stored and used as directed, the kit is stable for 1 year.

ADDITIONAL ITEMS NEEDED

1. Cell-free virus containing sample such as serum, plasma, whole blood, cell culture supernatant, nasal or throat swabs
2. Absolute ethanol
3. 1.5 ml nuclease-free (sterile) microfuge tubes
4. (Optional) RNAseOUT™ (Cat. #786-70) for cleaning working areas and equipment.
5. Nuclease free tips for the pipettes.

IMPORTANT INFORMATION

- GET™ Viral Nucleic Acid is available in 50 prep (Cat. # 786-1704) and 100 prep (Cat. # 786-1705) sizes with sample volume of 200 µl.
- Since the sensitivity and titer of potential pathogen (virus) varies with samples, the end user needs take appropriate safety measures when handling.
- Sterile handling of reagents and sample should be carried out to avoid contamination of reagents and sample with bacteria or nuclease. Frequent change of gloves and cleaning of work area with RNAseOUT™ (Cat. #786-70) is recommended. Avoid touching the mouth of reagent bottles.
- For performing RT-PCR, PCR, qPCR or qRT-PCR experiments using viral nucleic acid, ensure that sample preparation, RT-PCR or PCR run, and electrophoresis are carried out in separate work areas to avoid cross-contamination.

PREPARATION BEFORE USE

1. Add 18 ml of molecular grade ethanol to 30 ml GET Wash I bottle and check the box on the bottle label to indicate ethanol has been added.
2. Add 80ml molecular grade ethanol to the GET Wash II bottle (20 ml) and check the box on the bottle label to indicate ethanol has been added.
3. Equilibrate GET Elution Buffer to 60°C.
4. Briefly centrifuge the Poly A [Carrier RNA] tube. Add 250 µl of GET Elution Buffer to 1 vial of Poly A [Carrier RNA]. Gently mix with pipette to dissolve. Aliquot 50µl per microfuge vial and store the vials at -20°C as stock solution.
5. Store Longlife™ Proteinase K in small aliquots at -20°C for long term use. Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.
6. **Working solution of GET Binding Buffer** (*Prepare fresh and do not store after use*): Thaw one vial of Poly A [Carrier RNA] (50 µl). Transfer the Poly A [Carrier RNA] solution to 10 ml of GET Binding Buffer. Briefly vortex to mix.

PROTOCOL

Serum, plasma or cell culture supernatant viral samples

1. Add 200 µl of Genomic Lysis Buffer to 200 µl of viral sample. Vortex to mix.
   NOTE: *For sample volume <200 µl add PBS or 0.9%NaCl to make up the volume to 200µl.*
   NOTE: *For sample volume >200 µl increase the volume of reagents added proportionally.*
2. Add 10 µl Longlife™ Proteinase K suspension into the sample, mix and incubate at 55°C-60°C for 1 hr.
   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
   NOTE: *Do not exceed 60°C.*
3. Add 800 µl of working solution of GET Binding Buffer to the sample and vortex to mix
4. Transfer the 600 µl of the sample to a GET Silica column, positioned in a microfuge tube.
5. Centrifuge the column at 12,000x g for 1 minute at 25°C.
6. Discard the flow through.
7. Add the remaining 600 µl of the sample to the spin column and centrifuge at 12,000x g for 1 minute at 25°C.
8. Discard the flow through.
9. Apply 0.6ml Wash-I to the column and centrifuge at 12,000xg for 1 minute at 25°C. Discard the flow through.
10. Apply 0.6ml GET Wash II to the column and centrifuge at 12,000xg for 1 minute at 25°C. Discard the flow through.
11. Repeat step 10.

12. Centrifuge the column at 14,000xg for 3 minutes to remove residual GET Wash II buffer.
13. Discard the collection tube and place the column on a clean nuclease-free 1.5ml microfuge tube.
14. Add 25-50µl 50-60°C prewarmed GET Elution Buffer on top of the membrane in the column.
15. Incubate at room temperature for 15 minutes. Centrifuge the spin column at 12,000xg for 1 minute to collect the eluted DNA or RNA.
    NOTE*: Retain spin column until nucleic acid recovery is checked. If recovery is poor, add 25-50µl prewarmed (50-60°C) GET Elution Buffer to the column and repeat steps 14-15. Combine with previous elution.*
16. Store the eluted DNA at 4°C for short term use or at-20°C for long term use. Eluted RNA should be stored at -80°C.

Swab (Viral sample) from nose, throat or other test area

1. Transfer swab containing specimen sample (nose, throat or other test area) into a tube containing either 200 µl PBS or 0.9 % NaCl. Vortex the tube for 1-2 minutes to release the specimen (virus) in the solution.
2. Remove the swab and centrifuge the tube at 15,000 g for 10 minutes at room temperature.
   NOTE: *Alternatively, filtration can be used to remove cells from swabs.*
3. Add 200 µl Genomic Lysis Buffer. Briefly vortex to mix.
4. Add 10 µl Longlife™ Proteinase K suspension into the sample, mix and incubate at 55°C-60°C for 1 hr.
   NOTE: *Before use, Invert the Longlife™ Proteinase K tube 3-4 times to mix the enzyme suspension, then remove an aliquot for use.*
   NOTE*: Do not exceed 60°C.*
5. Add 800 µl of working solution of GET Binding Buffer to the sample and vortex to mix.
6. Transfer the 600 µl of the sample to a GET Silica column, positioned in a microfuge tube.
7. Centrifuge the column at 12,000x g for 1 minute at 25°C.
8. Discard the flow through.
9. Add the remaining 600 µl of the sample to the spin column and centrifuge at 12,000x g for 1 minute at 25°C.
10. Discard the flow through.
11. Apply 0.6 ml Wash-I to the column and centrifuge at 12,000xg for 1 minute at 25°C. Discard the flow through.
12. Apply 0.6 ml GET Wash II to the column and centrifuge at 12,000xg for 1 minute at 25°C. Discard the flow through.
13. Repeat step 13 once.
14. Centrifuge the column at 14,000xg for 3 minutes to remove residual GET Wash II buffer.

15. Discard the collection tube and place the column on a clean nuclease-free 1.5ml microfuge tube.
16. Add 25-50µl 50-60°C prewarmed GET Elution Buffer on top of the membrane in the column.
17. Incubate at room temperature for 15 minutes. Centrifuge the spin column at 12,000xg for 1 minute to collect the eluted DNA.
    ***NOTE**: Retain spin column until nucleic acid recovery is checked. If recovery is poor, add 25-50µl prewarmed (50-60°C) GET Elution Buffer to the column and repeat steps 17-18. Combine with previous elution.*
18. Store the eluted DNA at 4°C for short term use or at-20°C for long term use. Eluted RNA should be stored at -80°C.

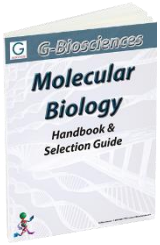
TROUBLESHOOTING

Issue	Suggested reason	Possible solution
Low nucleic acid yield or purity	Kit components are not stored properly	Store kit components as indicated in the label.
	Ethanol not added to the GET Wash I and II Buffers	Add absolute ethanol to GET Wash I and II Buffers before using
	Reagent and samples not properly mixed	Mix the sample tube well after addition of each reagent.
Low RNA yield	High levels of RNAse activity	Create RNase-free work environment. Use RNase OUT to clean the working bench
		Process starting material immediately or store at -80°C until it is processed
		Use eluted RNA directly for downstream application or store at -80°C for later use
	Incomplete Proteinase K digestion	Thaw Longlife™ Proteinase K on ice and Resuspend Proteinase K solution by inverting tube 3-4 times for uniform suspension before adding to the sample. Incubate for longer time if necessary.
Poor elution of nucleic acids	GET Elution Buffer provided in kit is not used	Use the GET Elution Buffer provided in the kit

		If using own water or GET Elution Buffer, ensure the pH of buffer is same as that of the GET Elution Buffer provided

RELATED PRODUCTS

Download our Molecular Biology Handbook.



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