GET™ Total RNA

Genomic Efficient Technology for purification of DNA-free RNA

(Cat. # 786-132)

INTRODUCTION

The *GET™* Total RNA kit is optimized to obtain highly pure total RNA from contaminating DNA, proteins, and nucleases.

GET™ Total RNA kit is based on our Genomic Efficient Technology (GET) for purification of RNA from various samples such as blood, animal tissue, plant tissue and yeast.

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 template 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 GET Elution Buffer (Fig.1). *GET™* Total RNA kit includes LongLife™ DNAse to remove the genomic DNA from purified nucleic acid pool to obtain DNA-free total RNA.

The eluted RNA is of high purity and is suitable for various downstream application processes such as Northern/slot/dot blots, reverse transcription or RNase protective assays. The kit supplied is suitable for 50 preps.

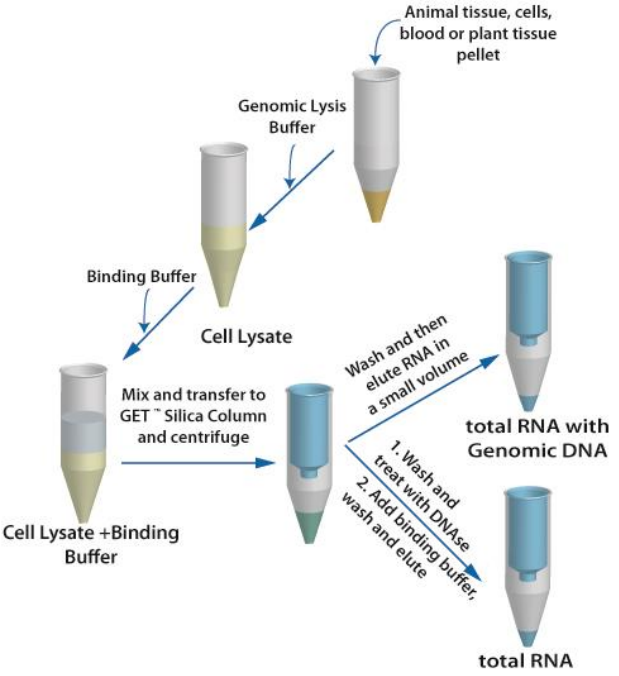


Fig:1

ITEM(S) SUPPLIED (CAT. # 786-132)

Description	50 preps
Genomic Lysis Buffer	30ml
GET Binding Buffer	50ml
Longlife™ Proteinase K	0.5 ml
LongLife™ DNAse	0.5 ml
GET Wash I	30ml
GET Wash II	20ml
GET Silica Columns	50
GET Elution Buffer	10ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components as indicated in the label. When stored as directed, the kit is stable for one year.

ADDITIONAL ITEMS REQUIRED

- 70% Ethanol, Absolute Ethanol,
- Nuclease free 1.5 ml microfuge tubes.
- **Optional**: Nucleic acid precipitation buffer (Cat. #786-058)

PREPARATION BEFORE USE

- 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.
- 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.
- Equilibrate GET Elution Buffer to 55°C to 60°C.

IMPORTANT INFORMATION

Every precaution must be taken to ensure that reagents and plastic ware are RNase-free and remain so while processing samples. Powder-free gloves must be worn at all times and changed frequently. It is recommended that all surfaces and equipment be liberally cleaned with RNaseOUT™ (Cat. # 786-70).

PROTOCOL

1a. ***Hand Grinding:*** Pipette 200 µl Genomic Lysis Buffer into a 1.5ml microfuge tube. Add 10-50mg tissue to the tube and grind tissues with a clean pestle or Dounce-type homogenizer. For grinding accessories see Application Note. Grind tissue until fully dispersed.

NOTE: *For optimal results, grind tissues to a powder in liquid nitrogen prior to adding to* Genomic Lysis Buffer.

1b. ***Mechanical Homogenization:*** Pipette 200 µl Genomic Lysis Buffer into a tube and homogenize with a polytron-type homogenizer.

1c. ***Cultured Cells:*** Add 200 µl Genomic Lysis Buffer per 1-2x10^6 cells trypsinized and pelleted cells. For cells grown in suspension, pellet cells, remove medium, and add 200 µl Genomic Lysis Buffer to the cell pellet. Do not wash the cells. Draw the cell lysate up and down several times with a narrow bore pipette tip to further disrupt the cells and shear DNA.

2. After homogenization, transfer the sample to an appropriate tube (if necessary).

3. Add 5 µl Longlife™ Proteinase K suspension into the sample and incubate at 55°C-60°C for 1 hr.
   NOTE: *Invert the Longlife™ Proteinase K tube 3-4 times to get uniform suspension before opening.*

4. Centrifuge the sample tube for 5 minutes at 5000 x g and transfer the clear supernatant to a clean tube. Add 400 µl of GET Binding Buffer and vortex to mix.

5. Transfer the sample into a GET Silica Column, positioned in a microfuge tube.

6. Centrifuge the column at 12,000 x g for 1 minute at 25°C.

7. Discard the flow through.

8. Apply 0.6 ml Wash-I to the column and centrifuge at 12,000xg for 1 minute at 25°C. Discard the flow through.

9. Apply 0.6 ml GET Wash II to the column and centrifuge at 12,000xg for 1 minute. Discard the flow through.

10. Repeat step 9.

11. After discarding the flow through from last wash, replace the spin column on the microfuge tube and spin at 14,000xg for 3 minutes to remove residual GET Wash II buffer.

12. Discard the collection tube and place the column on a clean nuclease-free 1.5ml microfuge tube.

13. Add 25-50µl 50-60°C warmed GET Elution Buffer on top of the membrane in the column.

14. 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 RNA recovery is checked. If recovery is poor, add 25-50µl prewarmed (50-60°C) GET Elution Buffer to the column and repeat steps 12-13. Combine with previous elution.*

OPTIONAL PROTOCOL

For DNA FREE RNA

15. Add 50µl GET Elution Buffer directly to the membrane in the spin column (Step-13). Add 5 µl LongLife™ DNAse and incubate the column at 37°C for 30 minutes.

16. Add 400µl GET Binding Buffer to the column and centrifuge the spin column at 12,000 x g for 1 minute. Discard the flow through from the collection tube and return the column to the same collection tube.
17. Apply 0.6 ml GET Wash I to the column and centrifuge the spin column at 12,000 x g for 1 minute. Discard the flow through from the collection tube and return the column to the same collection tube.
18. Apply 0.6 ml GET Wash II to the column and centrifuge the spin column at 12,000g for 1 minute. Discard the flow through.
19. Repeat the GET Wash II step (step 18). After the final wash, replace the spin column on the microfuge tube and centrifuge at 14,000 x g for 3 minutes to remove residual wash.
20. Transfer the spin column to a nuclease free microfuge tube. Add 50µl prewarmed (50-60°C) GET Elution Buffer on top of the membrane in the column. Incubate for 15 minutes at room temperature.
21. Centrifuge the sample at 12,000 x g for 1 minute to collect the RNA.

PRECIPITATION AND CONCENTRATION OF RNA

Some samples may require further precipitation to concentrate the RNA. After RNA is eluted, add 1/10th volume any nucleic acid precipitation buffer (not provided in kit, Cat. # 786-058) and 2 volumes of ethanol to the sample. Store at 4° C (or lower) for at least 15 minutes before using centrifugation to pellet RNA. Centrifuge at 14,000 x g for 5 minutes at 4°C to pellet RNA. Wash the RNA pellet with 70% ethanol.

REMOVAL OF CONTAMINATING DNA

DNA removal Following Isolation Procedure: As with any RNA isolation method, in some tissue samples, some residual DNA remains. Since PCR can essentially amplify a single DNA strand, there is no method of RNA purification that always guarantees complete removal of genomic DNA. Some applications are affected by DNA contamination. Therefore, we recommend removal of DNA with DNase I. For removing DNA, incubate 1 µg RNA prep with 1 U of DNase I for 30 minutes at 37°C, followed by heat-denaturation of the enzyme DNase I at 75°C for 5 minutes. DNase treatment can be performed in the same reaction tube in which the reverse transcription of mRNA to cDNA will eventually take place. For an examination of this procedure see, Huang et al., BioTechniques 20:1012-1020 (1996).

APPLICATION NOTES

Homogenization Techniques:

For efficient grinding of small samples, we offer Molecular Grinding Resin™ (Cat. # 786-138). Molecular Grinding Resin consists of high tensile micro particles that do not bind nucleic acids and allow most samples to be processed by hand using inexpensive macro centrifuge tube pestles or a mortar and pestle. The danger of grinding in liquid nitrogen is eliminated for many preparations.

Animal tissues: Several different methods can be used to isolate RNA from animal tissues. Perhaps the best is to use a polytron-type generator; tissues are disrupted nearly instantaneously, and genomic DNA is sheared allowing clean RNA preparation. Many investigators have also successfully isolated RNA using mortar and pestle or Dounce homogenizers. The use of Molecular Grinding Resin™ will greatly enhance yields with this method. Frozen tissues should be flash frozen in liquid nitrogen and stored at -70° C.

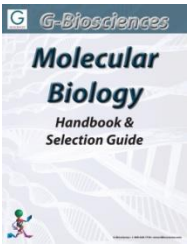
Plant and fungal tissues: Most plant and fungal tissues are best prepared by freezing as described above. Pulverize while frozen to a fine powder and quickly add the appropriate amount of Genomic Lysis Buffer followed by proteinase K treatment and addition of GET Binding Buffer.

Bacterial cells: For <5x10^8 Gram negative or Grampositive cells, we recommend lysis with lysozyme prior to RNA purification. Pellet cells and resuspend the pellet in 100µl TE buffer supplemented with 1mg/ml lysozyme. Incubate at room temperature for 5 minutes, with vortexing every 1-2 minutes. Add Genomic Lysis Buffer followed by Proteinase K treatment and addition of GET Binding Buffer.

Human Blood: For human blood, we recommend first lysing red blood cells with our RBC Lysis Buffer (Cat. # 786-649). Use a maximum volume of 1.5ml blood per column.

RELATED PRODUCTS

Download our Molecular Biology Handbook.



http://info.gbiosciences.com/complete-molecular-biology-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

Upgraded: 5/28/20

www.GBiosciences.com