# GET™ Total RNA-Mag

## Genomic Efficient Technology for purification of DNA-free RNA with silica magnetic beads

## (Cat. # 786-1727)

## INTRODUCTION

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

*GET*™ Total RNA-Mag 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 surface. Following the capture of nucleic acids on the silica membrane or beads, a series of washing steps removes interfering impurities. In the final step, pure nucleic acid is eluted in concentrated form with GET Elution Buffer.

The *GET*™ Total RNA-Mag kit is based upon the principle of binding of nucleic acids on silica beads with magnetic core in presence of chaotropic salts. Nucleic acids are bound to the silica magnetic beads under high concentrations of chaotropic salts and the impurities are removed during the wash steps (Fig:1). The purified nucleic acids are eluted with GET Elution Buffer. Furthermore, the kit includes LongLife™ DNAse to remove the genomic DNA from purified nucleic acid pool to obtain DNA-free total RNA. The RNA extraction is rapid with less than 15-30 minutes hands- on time.

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.

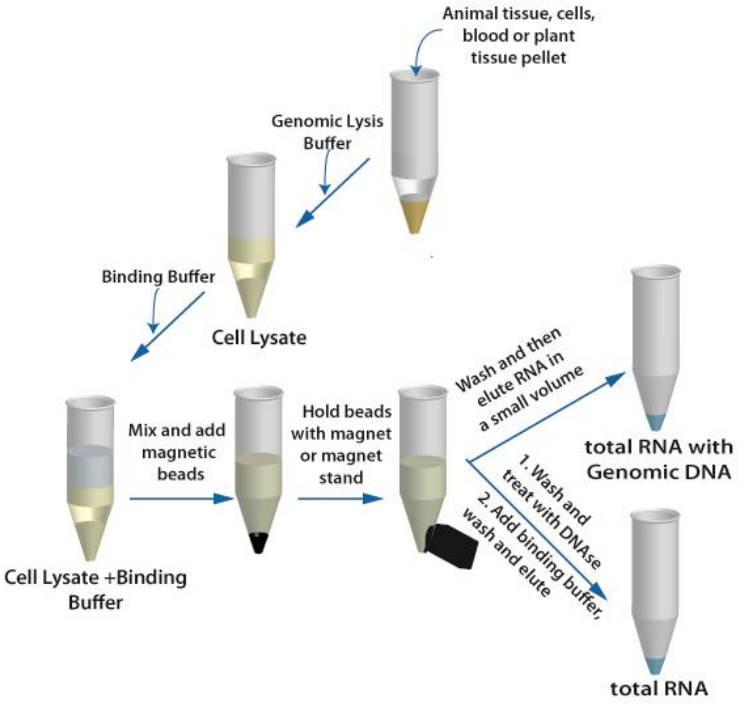



Fig:1

## ITEM(S) SUPPLIED (Cat. # 786-1727)

| 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 |
| Silica Magnetic Beads | 2.5 ml |
| 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.
- 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 1-2x$10^6$ 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. Add 50 µl of Silica Magnetic Beads to sample tube and gently invert 4-5 times to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
   **NOTE**: *Do not vortex or shake the sample containing silica magnetic beads vigorously.*
6. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
7. Gently remove and discard the supernatant without disturbing the beads.
8. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.
9. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
10. Gently remove and discard the supernatant without disturbing the beads.
11. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
12. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
13. Gently remove and discard the supernatant without disturbing the beads.
14. Repeat step 11-13.
15. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
16. Add 50 µl of prewarmed GET Elution Buffer to the magnetic beads and resuspend the beads complex by brief vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
17. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the supernatant in a 1.5 ml nuclease free tube.
    ***NOTE**: Check the RNA for recovery. If recovery is poor, add 25-50µl prewarmed (50-60°C) GET Elution Buffer to the magnetic beads and repeat steps 16-17. Combine with previous elution.*

## OPTIONAL PROTOCOL

***For DNA FREE RNA***

18. At the elution step, add 5 µl LongLife™ DNAse along with 50 µl of prewarmed GET Elution Buffer to the magnetic beads (Step 16) and resuspend the beads complex by brief vortex or shaking and incubate the tube at 37°C for 30 minutes.
19. Add 400µl GET Binding Buffer to tube and vortex to mix. Incubate the sample for 5 minutes with gentle mixing on rotor at room temperature.
20. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
21. Gently remove and discard the supernatant without disturbing the beads.
22. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash I to the sample tube and invert the tube gently 4-5 times to mix.

23. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
24. Gently remove and discard the supernatant without disturbing the beads.
25. Remove the tube from magnetic stand or magnet and add 0.5ml GET Wash II to the tube and invert the tube gently 4-5 times to mix.
26. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube.
27. Gently remove and remove the supernatant without disturbing the beads.
28. Repeat step 25-27.
29. Ensure all the liquid is removed from the magnetic beads. Air dry the beads for 5-20 minutes.
30. Add 50 µl of prewarmed GET Elution Buffer to the magnetic beads and resuspend the beads complex by brief vortex or shaking. Incubate for 15 minutes with gentle mixing on rotor at room temperature.
31. Place the sample tube on magnetic stand or use magnet to immobilize the beads at one end of the tube. Transfer the supernatant in a 1.5 ml nuclease free tube.
32. Store the eluted RNA at -80°C

## 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 [Longlife™ DNase]. 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.

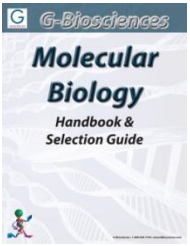
**Plant and fungal tissues**: Most plant and fungal tissues are best prepared by freezing as described above. Pulverize while frozen to a fine powder and quickly add the appropriate amount of Genomic Lysis Buffer followed by proteinase K treatment and addition of GET Binding Buffer.

**Bacterial cells**: For <5x$10^8$ 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 50 µl of Magnetic beads.

## RELATED PRODUCTS

Download our Molecular Biology Handbook.

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