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A Geno Technology, Inc. (USA) brand name

GET™ Total RNA

For Isolation of DNA-free RNA for RT-PCR

(Cat. # 786-132)

INTRODUCTION

The *GET*™ Total RNA kit isolates total RNA from contaminating DNA, proteins, and nucleases using our GET™ RNA Spin Columns. The 60-minute protocol is simple; after homogenization, RNA is bound to the GET™ RNA Spin Columns and washed. The protocol provides an option to remove contaminating DNA with a single DNase treatment. Finally RNA is eluted from the column. The eluted RNA is ready for any procedure including Northern/slot/dot blots, reverse transcription or RNase protective assays.

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

Description	Size
Arrest™ Extraction Buffer	60ml
RNA Precipitation Solution	10ml
GET™ RNA Spin Columns	50
Wash I	50ml
Wash II	2 x 20ml
RNA-Elution Buffer	3 x 1.5ml
DNase buffer	10ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store refrigerated. When stored and used properly, the kit is stable for one year.

ADDITIONAL ITEMS REQUIRED

70% Ethanol, Absolute Ethanol, Chloroform, 1M Dithiothreitol (DTT) solution (1000X), RNase-free DNase I (optional)

PREPARATION BEFORE USE

1. Add 80ml molecular grade ethanol to Wash II. Check the box on the label to indicate the ethanol has been added.
2. Equilibrate molecular grade water or RNA-Elution Buffer to 50-60°C.
3. Add DTT solution to Arrest™ Extraction Buffer required to make a 1X dilution (1µl 1M DTT/ 1ml Arrest™ Extraction Buffer). Only add to a volume of Arrest™ Extraction Buffer required.

IMPORTANT NOTE

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 300µl Arrest™ Extraction 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. Add an additional 300µl Arrest™ Extraction Buffer and vortex briefly to mix.

NOTE: *For optimal results, grind tissues to a powder in liquid nitrogen prior to adding to Arrest™ Extraction Buffer.*

1b. ***Mechanical Homogenization:*** Pipette 600µl Arrest™ Extraction Buffer into a tube and homogenize with a polytron-type homogenizer.

1c. ***Cultured Cells:*** Add 500µl Arrest™ Extraction Buffer per 1-2x10^6 cells. For attached cells remove culture medium and add Arrest™ Extraction Buffer directly to cells. For cells grown in suspension, pellet cells, remove medium, and add Arrest™ Extraction 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). Incubate for 5 minutes at room temperature to allow complete tissue lysis.
3. Add 60µl RNA Precipitation Solution and 200µl chloroform. Invert several times to mix. Centrifuge at 14,000-16.000g for 2 minutes to pellet the cell debris.
4. Carefully pipette the clear supernatant to a clean tube. Add 600µl 70% ethanol to the sample.
5. Transfer the sample to a GET™ RNA Spin Column and incubate at room temperature for 1-2 minutes.
6. Centrifuge the spin column at 14,000-16,000g for 1 minute. Discard the flow through from the collection tube and return the column to the same collection tube.
7. Add 500µl Wash I to the column and centrifuge the spin column at 14,000-16,000g for 1 minute. Discard the flow through from the collection tube and return the column to the same collection tube.
8. Add 600µl Wash II to the column and centrifuge the spin column at 14,000-16,000g for 1 minute. Discard the flow through from the collection tube and return the column to the same collection tube.
9. Repeat the Wash II step (step 8) two more times. After the final wash, return the column to the collection tube and centrifuge at 14,000-16,000g for 1 minute to remove residual wash.

NOTE: *If DNA-free RNA is required, continue at step 12, otherwise proceed to step 10.*

10. Transfer the spin column to a clean tube. Add 50µl prewarmed (50-60°C) Elution Buffer or RNase-free Molecular Grade Water (Cat. # 786-72C). Incubate for 5 minutes at 50-60ºC.
11. Centrifuge the sample at 14,000-16,000g for 1 minute to collect the RNA.

OPTIONAL PROTOCOL

For DNA FREE RNA

12. Add 50µl DNase Buffer directly to the membrane in the spin column. Add 50 units of RNase-free DNase I and incubate at 37ºC for 30 minutes.
13. Add 600µl Arrest™ Extraction Buffer to the column and centrifuge the spin column at 14,000-16,000g for 1 minute. Discard the flow through from the collection tube and return the column to the same collection tube.
14. Add 600µl 70% ethanol to the column. Incubate at room temperature for 5 minutes and then centrifuge the spin column at 14,000-16,000g for 1 minute. Discard the flow through from the collection tube and return the column to the same collection tube.
15. Add 500µl Wash I to the column and centrifuge the spin column at 14,000-16,000g for 1 minute. Discard the flow through from the collection tube and return the column to the same collection tube.
16. Add 600µl Wash II to the column and centrifuge the spin column at 14,000-16,000g for 1 minute. Discard the flow through from the collection tube and return the column to the same collection tube.
17. Repeat the Wash II step (step 8) two more times. After the final wash, return the column to the collection tube and centrifuge at 14,000-16,000g for 1 minute to remove residual wash.
18. Transfer the spin column to a clean tube. Add 50µl prewarmed (50-60°C) Elution Buffer or RNase-free Molecular Grade Water. Incubate for 5 minutes at 50-60ºC.
19. Centrifuge the sample at 14,000-16,000g for 1 minute to collect the RNA.

PRECIPITATION AND CONCENTRATION OF RNA

Some samples may require further precipitation to remove excess salts or to concentrate the RNA. After RNA is eluted, add 1/10th volume Precipitation Solution (or other appropriate precipitation salt) and 2 volumes of ethanol to the sample. Store at 4º C (or lower) for at least 15 minutes before using centrifugation 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 RT of mRNA to cDNA will eventually take place. For an examination of this procedure see, Huang et al., BioTechniques 20:1012-1020 (1996).

PROTOCOL VOLUME SUMMARY GUIDE

Reagents	Micro (µl)	Large (ml)
Arrest™ Extraction Buffer	600*	5.0
Chloroform	200	1.6
Precipitating Solution	60	0.5
Wash I	500	2.5
Wash II	600	5.0
Elution Buffer	50	0.5
DNase Buffer	50	0.5

* Total volume; protocol may require 2 ½ volumes.

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.

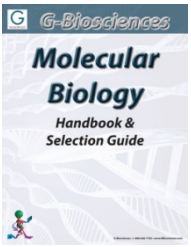
Plant and fungal tissues: Most plant and fungal tissues are best prepared by freezing as described above. Pulverize while frozen to a fine powder and quickly add the appropriate amount of Arrest™ Extraction Buffer.

Bacterial cells: For <5x10^8 Gram negative or Gram positive 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 300µl Arrest™ Extraction Buffer and mix by pipetting up and down. Continue at step 2 of main protocol.

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.



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