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A Geno Technology, Inc. (USA) brand name

Total Arrest™ RNA

For Isolation of DNA free RNA for RT-PCR

(Cat. #786-130, 786-131)

INTRODUCTION

The Total Arrest™ RNA isolates total RNA from contaminating DNA, proteins, and nucleases using our proprietary binding matrix, pinkRESIN™. Total Arrest™ RNA does not require phenol for extractions, making the kit one of the safest methods for isolating and purifying high quality RNA. The 60 minute protocol (micro kit) is simple; after homogenization, RNA is bound to pinkRESIN™ and washed. The protocol provides an option to remove contaminating DNA with a single DNase treatment. Finally, RNA is eluted from pinkRESIN™. The eluted RNA is ready for any procedure including Northern/slot/dot blots, reverse transcription or RNase protective assays. The kit is supplied in two formats, Micro for 50 isolations (10-50mg tissue each) and Large for 10 preps (100-500mg of tissue each).

ITEM(S) SUPPLIED

Description	Cat. #786-130, Micro	Cat. #786-131, Large
Arrest™ Extraction Buffer	60ml	100ml
RNA Precipitation Solution	10ml	10ml
*pink*RESIN™	3 x 1ml	5 x 1ml
Wash I	50ml	50ml
Wash II*	2 x 20ml*	3 x 20ml*
RNA-Elution Buffer	3 x 1.5ml	4 x 1.5ml
DNase buffer	10ml	10ml
Spin Columns	50 micro	10 large

**See Preparation Before Use*

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 ITEM(S) REQUIRED

- 70% Ethanol
- Absolute Ethanol
- Chloroform
- β-Mercaptoethanol
- RNase-free DNase I

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 Elution Buffer to 50-60°C.
3. Add 1µl β-Mercaptoethanol for every 1ml Arrest™ Extraction Buffer.
4. Depending on your choice of protocol, use a DNase I preparation.

CAUTION

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 RNase Out™ (Cat. # 786-70) or similar product.

All steps should be carried out at room temperature unless otherwise indicated.

PROTOCOL

NOTE: *The volumes given are for the Micro Total Arrest™ Kit; volumes for the Large Kit are given in parentheses. A summary of required volumes is provided on the back page. The protocol should be carried out in 1.5ml microfuge tubes or 50ml polypropylene centrifuge tubes depending on kit size.*

1. Grind the tissues or cells using one of the following procedures:
   a. Hand Grinding. Pipette 300µl (5ml) Arrest™ Extraction Buffer into 1.5ml microfuge tube. Add 10-50 mg (100-500mg) tissue to the tube and grind tissues with clean mortar and pestle or Dounce-type homogenizer. For grinding accessories see Appendix. Grind tissue with a microtube homogenizer (or mortar and pestle) until finely dispersed. Micro protocol users add 300µl additional Arrest™ Extraction Buffer to bring the total volume to 600µl. For optimal results, grind tissues to a powder in liquid nitrogen or on dry ice prior to adding to Arrest™ Extraction Buffer.
   b. Mechanical homogenization: Pipette 600µl (5ml) Arrest™ Extraction Buffer into a tube and homogenize with a polytron-type generator.
   c. Cells in culture: Add 500µl Arrest™ Extraction Buffer per 1-2 million cells. See Appendix for further instructions.
2. After homogenization, transfer the sample to an appropriate tube (if necessary). Let the sample incubate for 5 minutes at room temperature to complete tissue lysis.
3. Add 60µl (0.5ml) Precipitating Solution and 200µl (1.6ml) chloroform. Invert several times to mix. Centrifuge at 14,000g for 5-10 minutes to pellet the cell debris.
4. Carefully pipette the upper phase containing the clear supernatant to a clean tube. Add 600µl (5ml) 70% ethanol to the sample. Do not mix at this point.
5. Vigorously vortex the *pink*RESIN™ tube to re-suspend the resin. Pipette 50µl (500µl) *pink*RESIN™ to each tube using a large bore pipette tip. Invert to mix and incubate the sample at room temperature for 5 minutes. Keep the *pink*RESIN™ suspended by inverting the sample tube several times.
6. Centrifuge at 14,000g for 5-10 minutes to pellet the RNA bound to *pink*RESIN™ and decant and discard the supernatant. Tap or flick (do not vortex) the tube to re-suspend *pink*RESIN™ in the small, remaining volume of supernatant.
7. Add 0.5ml (2.5ml) Wash I to each tube and tap or flick the tube to re-suspend the *pink*RESIN™. Centrifuge to pellet the *pink*RESIN™ and decant off the Wash I. Invert the tube on a clean absorbent tissue to drain off all free Wash I.
   NOTE: *To remove contaminating DNA read the "Removal of Contaminating DNA" on the next page.*
8. Add 600µl (5ml) Wash II to each tube (use only Wash II that contains added ethanol) and tap the tube to re-suspend the *pink*RESIN™.
9. Place a spin column in a clean tube. Resuspend the *pink*RESIN™ and quickly decant or pipette into the spin column. Ensure that any *pink*RESIN™ remaining in the tube

is transferred into the spin column. Centrifuge the sample for 1-2 minutes at maximum speed and remove the Wash II from the lower tube.

10. Wash the *pink*RESIN™ pellet twice more using the same volume Wash II. Upon adding Wash II, gently flick or tap the tube/spin column to re-suspend the *pink*RESIN™. After removing the last wash from the lower tube, centrifuge a final time to remove any remaining wash from the bottom and sides of the spin column.
11. Transfer each spin column to a clean tube. Add 50µl (500µl) warmed (50-60°C) Elution Buffer or RNase-free Molecular Grade Water. Gently flick or tap the tube/spin column to re-suspend the *pink*RESIN™. Incubate for 5 min at 50-60°C. Centrifuge the sample for 1-2 minutes at maximum speed to collect the RNA

Precipitation and Concentration of RNA (Optional)

12. 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 4ºC (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 1µg RNA prep with 1U of DNase I for 30 minutes at 37^{o}C, followed by heat-denaturation of the enzyme DNase I at 75^{o}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.

Removal of Contaminating DNA During Isolation Procedure

1. Continue after step 7 of the main protocol.
2. Add 600µl (5ml) Wash II to each tube (use only Wash II that contains added ethanol) and tap or flick the tube to re-suspend the sample *pink*RESIN™. Centrifuge to pellet the *pink*RESIN™ and decant off the Wash II. Tap or flick the tube again to re-suspend the pellet. Repeat the washing with Wash II two more times. At each washing step, gently flick or tap the tube/spin column to re-suspend the *pink*RESIN™.
3. After the last wash, invert the tube on a clean absorbent tissue to drain off all free Wash II. Air-dry the tube for ten minutes.
4. Add 50µl (500µl) of DNase Buffer. Tap or flick the tube to re-suspend the resin. Add 50 units (500 units) of RNase-free DNase I. Tap or flick the tube briefly to re-suspend the *pink*RESIN™ and incubate at 37°C for 30 minutes.
5. Add 600µl (5ml) of Arrest™ Extraction Buffer to the *pink*RESIN™. Add 600µl (5ml) 70% ethanol. Incubate the sample at room temperature for 5 minutes. Keep the resin suspended by flicking the tube several times.
6. Centrifuge for 1-2 minutes at maximum speed to pellet the RNA bound *pink*RESIN™ and decant and discard the supernatant. Tap or flick (do not vortex) the tube to re-suspend the *pink*RESIN™ in the small and remaining volume of supernatant.
7. Return to the main protocol and continue at Step 7.

VOLUME SUMMARY GUIDE

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

APPENDIX

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

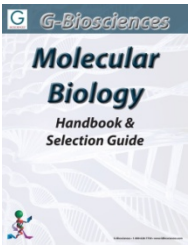
Most plant and fungal tissues are best prepared by freezing as described above. Pulverize while frozen to a fine powder and quickly add the appropriate amount of Arrest™ Extraction Buffer.

Cultured 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 extraction buffer to the cell pellet. Do not wash the cells. In both cases, draw the cell lysate up and down several times with a narrow bore pipette tip to further disrupt the cells and shear DNA.

RELATED PRODUCTS

Download our Molecular Biology Handbook.



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