GeneMark

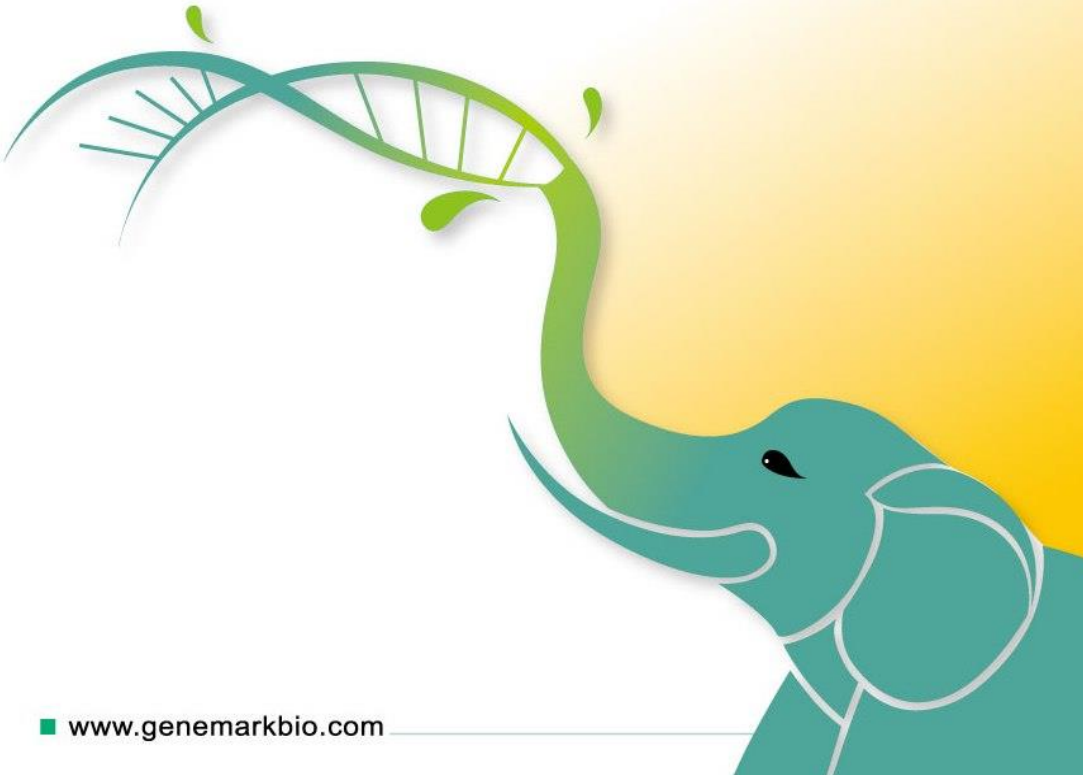
Total RNA Purification Kit

Cat. #.: **TR01 / TR01-150**

Size : **50 / 150** Reactions

Store at RT

For research use only



Description:

The **Total RNA Purification Kit** provides a rapid, simple and effective approach to isolate the total RNA from various animal/plant tissues, cultured cells and bacteria. The process is based on a spin column format. The procedure involves tissue or cells disruption and homogenization in the presence of guanidinium thiocyanate. After adding ethanol, RNA binds selectively to a silica membrane. Residual contaminating genomic DNA is digested by DNase I, which is applied directly in the column. After a series of rapid "wash-and-spin" steps to remove contaminating cellular components, the RNA remains bound to the membrane. Finally, nuclease-free water is used to elute the RNA from the membrane. Note that RNA shorter than 200 nt, such as 5S RNA, tRNA and microRNA, do not recover efficiently with this system.

Components of the Kit:

	TR01	TR01-150
1. RNA Lysis Solution	**35 ml (add 350 µl of 2-ME before use)**	**105 ml (add 1.05 ml of 2-ME before use)**
2. DNase I Solution 2 U/µl*	120 µl	120 µl x 3
3. DNase I Incubation Buffer	4.5 ml	13.5 ml
4. RNA Wash Solution I	55 ml	165 ml
5. RNA Wash Solution II	**16 ml (add 64 ml of Ethanol before use)**	**48 ml (add 192 ml of Ethanol before use)**
6. Nuclease-free water	10 ml	30 ml
7. RNA Spin Column with Collection Tube	25 sets x 2	25 sets x 6
8. Collection Tube	50 pcs	50 pcs x 3

** Store the DNase I Solution at -20 ℃; store all other kit components at room temperature (25~28°C).*

Materials to be supplied by the user:

- 2-Mercaptoethanol (2-ME): For preparing the RNA Lysis solution
- Rotor-stator homogenizer (e.g., PolyTron). Alternatively, mortar and pestle with syringe and 20G needle.
- 70% Ethanol (for animal tissues and cultured cells)
- 100% Ethanol (for preparing RNA Wash Solution II; for animal fibrous tissues, e.g., heart, muscle and skin; for RNA clean-up)
- Proteinase K (only for animal fibrous tissue, e.g., heart, muscle and skin)
- PBS buffer (optional for attached culture cells)
- Lysozyme (for bacteria strains)

Before starting this procedure:

- Add 350 µl (TR01) or 1.05 ml (TR01-150) of 2-Mercaptoethanol (2-ME) to the RNA Lysis Solution, and **store RNA Lysis/2-ME Solution at 4°C**.
- Add 64 ml (TR01) or 192 ml (TR01-150) of **100% Ethanol** to the RNA Wash Solution II.

Sample Preparation

A. Tissue Culture Cells

Table 1: RNA Lysis/2-ME Solution volume for cell preparation

Sample Size*	RNA Lysis/2-ME Solution
Pelleted Cells	
< 5 x 10^6	350 µl
5 x 10^6 ~ 1x 10^7	600 µl
Attached Cells	
dish diameter < 6 cm	350 µl
dish diameter < 10 cm	600 µl

** Do not use too much sample, to prevent clogging of column, resulting in much lower yield and quality.*

a. Cells Grown in Suspension (< 10^7)

(1) Pellet cells for 5 min at 300 x *g* and remove the medium **completely**.

(2) Vortex or flick the tube to loosen cells and add the appropriate volume of **RNA Lysis/2-ME Solution** (see Table 1 above) to the pelleted cells.

(3) Mix by vortexing or pipetting thoroughly until clump disappears.

(4) Homogenize the cells for 30 sec by rotor-stator or pass the lysate through a 20G needle at least 5~10 times or until a homogenous lysate is formed, without a sticky appearance.

(5) Add an equal volume of 70% Ethanol to the lysate and mix well by vortexing or pipetting. Continue to **the RNA Isolation** Section (page 8).

b. Attached Cells

Two methods can be used for sample treatment:

- Lysing the cells directly in a culture dish

  Remove medium **completely** and add the appropriate volume of **RNA Lysis/2-ME Solution** (see Table 1 above) to the dish. Immediately tip the dish to completely cover the cells, collect the lysate with a rubber policeman and follow steps (3) to (5) as per **Cells Grown in Suspension protocol**. Alternatively, without a rubber policeman, incubate for 2 min then repeat rocking the dish and transfer the lysate to a 1.5 ml microcentrifuge tube. Follow steps (3) to (5) as per **Cells Grown in Suspension protocol**.

- Trypsinizing the cells before lysis

  Remove medium **completely** and wash with PBS, then remove PBS and add 0.1%~0.25% trypsin. After the cells have detached from the dish, transfer the cells to a 1.5 ml microcentrifuge tube and centrifuge at 300 x *g* for 5 min. Completely remove the supernatant and follow steps (2) to (5) as per **Cells Grown in Suspension protocol**.

B. Animal Tissue

Note: For best quality and yield of RNA, harvest tissue immediately from a freshly sacrificed animal. Alternatively, tissue may be immediately snap-frozen in liquid nitrogen and stored at -70°C or tissue can be stabilized in RNAfter™ reagent (GeneMark Cat. No. RA-100). The sample can be stored in RNAfter™ reagent for a day at 37°C, 1 week at room temperature (25~28°C), one month at 4°C and indefinitely at -20°C. The purified RNA quality is as high as those stored in liquid nitrogen.

Note: For fibrous tissue (e.g., heart, muscle and skin), use the modified RNA isolation protocol (Appendix II), which includes a proteinase K digestion step to facilitate cell disruption.

Table 2: RNA Lysis/2-ME Solution volume for tissue lysis

Sample Size*	RNA Lysis/2-ME Solution
< 20 mg	350 µl or 600 µl*
20~30 mg	600 µl

** Do not use too much sample to prevent clogging of column, resulting in much lower yield and quality.*

** For spleen, thymus, lung or other hard-to-lyse tissue, use 600 µl of **RNA Lysis/2-ME Solution** for < 20 mg starting sample material.*

(1) Quickly slice and weigh fresh, frozen or RNAfter™ reagent stabilized tissue and place into a suitable sized vessel for homogenization.

** Do not allow the frozen sample to thaw during weighing before adding the RNA Lysis/2-ME Solution.*

(2) Immediately homogenize the samples using one of the following methods:

a. Rotor-Stator homogenization:

Add the appropriate volume of **RNA Lysis/2-ME Solution** (see Table 2 above) to the sample, then homogenize it thoroughly until uniform without a viscous solution being formed (usually for 20~60 sec, depending on tissue type).

b. Mortar and pestle with 20G needle and syringe homogenization:
Freeze the sample immediately in liquid nitrogen and grind it into a fine powder under liquid nitrogen. Transfer the sample powder into a **pre-chilled** tube and add the appropriate volume of **RNA Lysis/2-ME Solution** (see Table 2 above). Homogenize the lysate thoroughly by passing it through a 20G syringe needle until uniform and without a viscous solution being formed (usually >10 times, depending on tissue type).

***** *Mortar and pestle is used only for disruption; the lysate needs to be further homogenized and genomic DNA needs to be sheared by passing it through a syringe needle. Otherwise, RNA yield and quality will be significantly reduced.*

** Do not use a needle thinner than 20G (>20G, for example 23G), which may clog during the process of passing the lysate through.*

(3) Spin the homogenous lysate at top speed for 3 min. Carefully transfer the supernatant to a new tube without pipetting or disturbing the pellet.

** It is important to avoid carry-over of the pellet, which may clog the column and produce much lower yield and quality*.

** If the supernatant is still viscous, the pellet may be transferred during pipetting. This indicates the homogenization is incomplete and needs to be homogenized again by a rotor-stator or 20G syringe needle.*

(4) Add an equal volume of **70% Ethanol** to the lysate and mix well by vortexing or pipetting. Continue to the **RNA Isolation** Section (page 8).

C. Bacteria sample

Note: Before starting, prepare fresh lysozyme solution in TE buffer. Prepare 400 µg/ml for Gram-negative bacteria and 3 mg/ml for Gram-positive strain.

(1) Harvest bacteria (< 1 x 10^9) by spinning at top speed for 2 min. **Completely** remove the medium by pipetting.

(2) Vortex or flick the tube to loosen the pellet, add 100 µl freshly prepared lysozyme to the pellet and vortex to resuspend the cells.

(3) Incubate the resuspended pellet at room temperature **(25~28*°C*).** For Gram-negative bacteria, incubate for 3~5 min; for Gram-positive bacteria, incubate for 5~10 min.

(4) Add 350 µl **RNA Lysis/2-ME Solution** to the sample and mix by vortexing. Spin at top speed for 2 min and transfer the supernatant to a clean tube without pipetting and disturbing the pellet.

(5) Add 250 µl of 100% Ethanol to the lysate and mix well by vortex or pipetting. Continue to the **RNA Isolation** Section (page 8).

D. Plant tissue

The Plant Total RNA Purification Kit (GeneMark Cat. No. TR02) is recommended for plant samples, since it can be applied for a broader range of plant samples.

The protocol in this manual may only work just for some plant tissues.

Note: For best quality and yield of RNA, harvest tissues immediately from a plant. Alternatively, tissues may be immediately snap-frozen in liquid nitrogen and stored at -70°C or can be stabilized in RNAfter™ reagent. The sample can be stored in RNAfter™ reagent for a day at 37°C, 1 week at room temperature**(25~28*°C*)**, one month at 4°C and indefinitely at -20°C.

Note: This kit cannot be used for plant tissues with high levels of starch, phenols and secondary metabolites (such as some woody trees, milky endosperm or mycelia of filament fungi) since **RNA Lysis/2-ME Solution** will become solidified or very viscous after addition to sample powder. Please use **Plant Total RNA Purification Kit** for such applications.

(1) Quickly weigh no more than 100 mg fresh, frozen or RNAfter™ reagent stabilized tissue and grind it into a fine powder under liquid nitrogen.
** Do not allow the frozen sample to thaw during weighing before adding RNA Lysis/2-ME Solution.*

(2) Immediately transfer the powder (<100 mg) into a tube and add 450 µl of RNA Lysis/2-ME Solution, then vortex vigorously and incubate at 60°C for 3 min.
** For samples with high starch content, omit the 60°C incubation step, otherwise the volume may increase considerably at high temperature.*

(3) Spin the homogenous lysate at top speed for 3 min. Carefully transfer the supernatant to a new tube without pipetting and disturbing the pellet.
** It is important to avoid carry-over of the pellet, which may clog the column and produce much lower yield and quality.*

(4) Add 0.5 volumes of 100% Ethanol to the lysate and mix well by pipetting. Continue to the **RNA Isolation** Section (page 8).

RNA Isolation

This is a continuation of the procedures from Sample Preparation in Sections A, B, C and D.

1. Load up to 700 µl of lysate/ethanol mixture into an **RNA Spin Column** inserted in a 2 ml **Collection Tube**, then spin at top speed (12,000~14,000 x*g*) for 1 min and discard the flow-through.
   ** If the volume of lysate/ethanol mixture is greater than 700 µl, apply any remaining lysate/ethanol mixture into the column and repeat the centrifugation step once more.*
2. Transfer the RNA Spin Column into the original collection tube and add 500 µl **RNA Wash Solution I**, then spin at top speed for 1 min and discard the flow-through.
3. **DNase I digestion.**
   For each isolation reaction , premix 80 µl **DNase I Incubation Buffer** with 2 µl **DNase I** in a new sterile tube (Mix by flicking or inverting the tube, do not vortex!). Add 82 µl of the solution into the **center** of the RNA Spin Column membrane and incubate at room temperature (25~28℃) for 15 min.
   ** If processing multiple samples at a time, prepare a fresh mixture of DNase I solution just before use, do not storea premix of DNase I solution.*
4. Add 500 µl of **RNA wash solution I** to the RNA Spin Column, spin at top speed for 1 min and discard the flow-through.
5. Place the RNA Spin Column to the original collection tube. Add 600 µl **RNA Wash Solution II**, spin at top speed for 1 min and discard the flow-through. Repeat this step once more.
6. Place the RNA Spin Column into the collection tube and spin at top speed for 3 min to remove any residual ethanol.
   ** If the centrifugation speed is lower than 12,000 xg or residual ethanol from RNA wash solution II must be removed completely, incubate the*

RNA Spin Column at 60~65°C in a drying oven for 5 min to evaporate all of the remaining ethanol.

7. Place the RNA Spin Column in a clean 1.5 ml microcentrifuge tube. Add 30~50 µl of **Nuclease-free water** into the center of the RNA Spin Column membrane and let stand for 1 min. Centrifuge for 1 min at top speed to elute the RNA and store the RNA sample at -70°C.

Troubleshooting Guide

Problem	Solutions
Column is clogged	
a) Too much starting material.	Use the correct amount of starting material. Make sure the lysate is sufficiently disrupted and homogenized.
b) Sample lysate contains insoluble residues. (Centrifugation before adding ethanol not performed)	After sample lysis (except for tissue culture cells), centrifuge the sample at top speed for 3 min or longer and only use the supernatant.
Poor RNA yield	
a) Too much starting material.	It is essential to use the appropriate amount of starting material.
b) Tissues or cultured cells are too old.	Use cultures before they reach maximum density or become fully confluent, and harvest tissues as rapidly as possible.
c) Insufficient disruption or homogenization	1) Decrease the amount of starting material. 2) Perform lysis and homogenization as recommended for each sample type using the appropriate lysis buffer as recommended. 3) Cut tissue samples into smaller pieces and homogenize them in the RNA Lysis/2-ME Solution until fully dissolved.
d) RNA still bound to the membrane	Repeat elution, but incubate the column at room temperature for 10 min with Nuclease-free water before centrifugation.

RNA is degraded	
a) RNase contamination	1) Use RNase-free solution, pipette tips, plastic-ware and glassware. 2) Wipe laboratory environment with RNase DNase Away Solution (GeneMark Cat. No. D0339). 3) Change gloves frequently.
b) Improper handling of sample from harvest until lysis	1) If not processed immediately, snap-freeze tissue immediately after harvesting and store at -70°C or in liquid nitrogen. 2) Samples must remain frozen until RNA Lysis/2-ME Solution is added. 3) Tissues may be stored in RNAfter™ reagent(GeneMark Cat. No. RA-100)
DNA contamination	
a) No DNase I treatment.	Be sure to add 2 µl DNase I (supplied with DNase I Incubation Buffer).
b) No incubation with RNA Wash Solution I before DNase I treatment.	Be sure to treat spin column with RNA Wash Solution I in step 2 before DNase I digestion.
RNA does not perform well in downstream experiments	
a) Ethanol carry-over	Be sure to dry the membrane by centrifugation at > 12,000 x*g* for 3 min. Following the centrifugation, incubate the

	RNA Spin Column at 60~65°C in a drying oven for 5 min to evaporate all of the remaining ethanol.
b) Residual salt in eluate	Residual guanidine thiocyanate will also inhibit enzyme activities. Transfer the RNA Spin Column to a clean 1.5 ml microcentrifuge tube before adding RNA Wash Solution II.

Appendix I: Protocol for RNA Clean-Up or Genomic DNA Removal

This kit can be used to clean up RNA or to remove genomic DNA contamination for RNA samples purified using different isolation methods.

1. Adjust RNA volume to 100 µl with Nuclease-free water. Add 350 µl of **RNA Lysis/2-ME Solution** and mix well.
2. Add 250 µl of 100% Ethanol to the lysate and mix by pipetting. Continue to the **RNA Isolation** Section (page 8).

Appendix II: Protocol for Isolation of Total RNA from Heart, Muscle and Skin Tissues

Additional reagent and equipment required:

- Proteinase K (20 mg/ml)
- Water bath or heating block at 56°C

Procedures:

1. Process and homogenize the sample as per **Sample Preparation Section B Animal Tissue** (page 4) procedures (1) to (2). Add 590 µl Nuclease free water to the homogenate, then add 10 µl proteinase K and mix by pipetting.
2. Incubate at 56°C for 10 min.
3. Spin the lysate at top speed for 3 min and carefully transfer the supernatant to a new tube without pipetting and disturbing the pellet.
4. Add 0.5 volumes of 100% Ethanol to the lysate and mix by pipetting. Continue to the **RNA Isolation** Section (page 8).

Appendix III: Protocol for Isolation of Total RNA from Yeast

Additional reagents required:

- Lyticase or zymolase
- Sorbitol buffer: For enzymatic lysis, prepare Sorbitol buffer containing 1 M Sorbitol, 0.1 M EDTA (pH 7.4). Add 0.1% 2-mercaptoethanol and lyticase/zymolase just before use.

Procedures:

1. Pellet fresh yeast cells (<5 x 10^6) at 5000 x *g* for 5 min at 4°C and remove the medium completely.
2. Resuspend the cells in 1 ml freshly prepared Sorbitol Buffer containing lyticase or zymolase (final concentration of 50 U per 1 x 10^7 cells). Incubate for 10~30 min at 30°C with gentle shaking to generate spheroplasts.
3. Spin down the spheroplasts at 300 x *g* for 5 min and carefully remove the supernatant.
4. Add 350 µl of **RNA Lysis/2-ME Solution** to lyse the spheroplasts and mix by vortexing vigorously. Spin at top speed for 2 min and transfer the supernatant to a clean tube without pipetting or disturbing the pellet.
5. Add 350 µl of 70% Ethanol to the lysate and mix well by vortexing or pipetting. Continue to the **RNA Isolation** Section (page 8).

Appendix IV: Protocol for Isolation of Total RNA from Fresh Blood Samples

Additional reagents and equipments required:

- 10X RBC Lysis Solution (GeneMark Cat. No. DP023-RBC: 10X RBC Lysis Solution, dilute to 1X RBC Lysis Solution before use)

Procedures:

1. Transfer the fresh blood sample **(<0.5 ml)** to the centrifuge tube and add 8 volumes of **1X RBC Lysis Solution**. Mix by inverting and incubate at room temperature for 10 min or longer until red blood cells are completely lysed (solution becomes clear red); invert the tube 2~3 times during incubation.
2. Centrifuge at 3,000 x *g* for 5 min and remove the supernatant without pipetting or disturbing the pellet.
3. Resuspend and wash the leukocyte pellet in 2 volumes of **1X RBC Lysis Solution**. Mix well by vortexing.
4. Centrifuge for 3,000 x *g* for 5 min and remove the supernatant without pipetting or disturbing the pellet.
5. Vortex or flick the tube to loosen the cells and add 350 µl of **RNA Lysis/ 2-ME Solution**.
6. Vortex or pipette thoroughly until there are no clumps and solution appears clear and homogenous.
7. Homogenize the cells for 30 sec by rotor-stator or pass the lysate through a 20G needle at least 5~10 times or until the lysate becomes homogenous, without a sticky appearance.
8. Add 350 µl of 70% Ethanol to the lysate and mix well by vortexing or pipetting. Continue to **the RNA Isolation** Section (page 8).

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