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Product Information

GenElute™ 96 Well Total RNA Purification Kit

Catalog Number **RTN9604**

TECHNICAL BULLETIN

Product Description

The GenElute™ 96 Well Total RNA Purification Kit provides a simple and convenient way to isolate total RNA from cells or tissue. The kit utilizes the advantages of silica membrane technology and eliminates the use of hazardous organic compounds such as phenol and chloroform.

The cells or tissue are lysed by incubation in a solution containing large amounts of a chaotropic salt. This RNA Lysis Solution immediately inactivates RNases which are present in virtually all biological materials. The RNA Lysis Solution and RNA Wash Buffer 3 create the binding conditions which are necessary to facilitate adsorption of RNA to the silica membrane. The contaminating DNA that is bound to the silica membrane is removed by directly applying RNase-free DNase. The remaining salts, proteins, and other cellular debris are removed by simple washing steps. Finally, pure RNA is eluted under low ionic strength conditions with RNase-free water which is supplied in the kit.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

RNA Lysis Solution and RNA Wash Buffer 1 contain guanidinium salt which is an irritant so gloves and safety glasses should be worn.

Storage/Stability

Store lyophilized rDNase, RNase-free, Catalog Number R2783, at 2–8 °C on arrival (stable up to 1 year).

Store the remaining components of the GenElute 96 Well Total RNA Purification Kit at room temperature. If any regent forms a precipitate, warm at 30–40 °C until the precipitate dissolves and allow to cool to room temperature before use. All reagents are stable for at least one year when stored properly.

Components

Reagents Provided	Catalog Number	RTN9604 4 × 96 Preps
RNA Lysis Solution	L7170	125 mL
RNA Wash Buffer 1	W3395	360 mL
RNA Wash Buffer 2, Concentrate	W3520	2 × 90 mL
RNA Wash Buffer 3, Concentrate	W3645	2 × 65 mL
Reaction Buffer for rDNase	R2658	4 × 30 mL
rDNase, RNase-free	R2783	8 VL
RNase-free Water	R2908	2 × 65 mL
RNA Binding Plates, blue ring	R3033	4 Each
Collection Tubes, 1.5 mL	T8330	16 Each
Wash Plates	W3270	4 Each
Square-well Blocks	S9326	6 Each
Elution Plate, U-Bottom	E5911	4 Each
Round-well Blocks Low	R3408	4 Each

Reagents and Equipment Required but Not Provided.

- GenElute Vacuum Manifold, Cat No. VM01
- Vacuum Source (vacuum of 200–400 mbar)
- GenElute Vacuum Regulator, Cat No. VM02 (recommended)
- 95-100% Ethanol, Cat No. E7148, E7023, or 459836
- Centrifuge capable of 5,600–6,000 × *g* (optional)
- 2-mercaptoethanol, Cat No. M3148
- Filter Plate, Cat No. VM03 (optional)

Preparation Instructions
Before beginning the procedure, complete the following:

1. Reconstitute rDNase, RNase-Free by adding 540 µL of RNase-free Water to the rDNase vial and incubate at room temperature for 1 minute. Gently mix the vial contents to ensure the rDNase dissolves completely. If not using the entire 96 well plate, dispense the reconstituted rDNase solution into aliquots and store at −20 °C for up to 6 months. Do not freeze / thaw the aliquots more than three times.

2. Prepare rDNase reaction mixture: For each sample to be processed mix 10 µL of reconstituted rDNase with 90 µL of Reaction Buffer for rDNase.

3. RNA Wash Buffer 2: Add 360 mL of 96-100% ethanol to each 90 mL bottle of RNA Wash Buffer 2, Concentrate. Mark the label of the bottle to indicate that ethanol is added. After each use, tightly cap the diluted RNA Wash Buffer 2 to prevent ethanol evaporation.

4. RNA Wash Buffer 3: Add 150 mL of 96-100% ethanol to the RNA Wash Buffer 3, Concentrate. Mark the label of the bottle to indicate that ethanol is added. After each use, tightly cap the diluted RNA Wash Buffer 3 to prevent ethanol evaporation.

Vacuum Manifold Use
Establish a reliable vacuum source for the GenElute Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. A vacuum of 200-400 mbar (pressure difference) is recommended. The use of a GenElute Vacuum Regulator (VM02) is also recommended. Alternatively, adjust vacuum during the purification such that the sample flows through the column with a rate of 1-2 drops per second.

Procedures
Cell Preparation
If using a vacuum, up to 2×10^6 cells can be processed and if using centrifugation, up to 1×10^7 cells can be pelleted.

1. *Harvest cells*. If cells are grown in suspension, aliquots of up to 2×10^6 cells can be transferred into Tube Strips (included in the kit) or the wells of another suitable deep-well plate. Pellet cells by centrifugation for 5 minutes at 500 × *g* and remove the supernatant by pipetting.

2. *Lyse cells.*
   - *For pelleted cells*, add 300 µL of RNA Lysis Buffer and 3 µL of 2-mercaptoethanol to each sample. Cells can be lysed by pipetting up and down repeatedly, or by vigorous shaking of the sealed/closed plate or reaction tube.
   - *For attached cells*, make sure the culture medium is completely removed. Lyse cells by the addition of 130 µL of RNA Lysis Buffer and 1.3 µL of 2-mercaptoethanol to each well of a cell culture plate. Following lysis add 130 µL of RNA Wash Buffer 3 to adjust the RNA binding conditions.

If using more than 1×10^6 cells, it is recommended to use a commercial homogenizer after lysis with RNA Lysis Buffer to reduce the viscosity. To prevent the RNA Binding Plate from clogging it is recommended to filter the lysate through the Filter Plate (VM03, not included) before applying the lysate to the RNA Binding Plate.

Tissue Preparation
For best yields of intact RNA, harvest tissue as quickly as possible from a freshly sacrificed animal and proceed with the following procedure immediately. Alternatively, tissue may be immediately flash-frozen in liquid nitrogen and stored at –70 °C for several months prior to RNA extraction, or the tissue may be stabilized in RNA*later*®, Catalog Number R0901. RNA*later* stabilized tissue can be stored for up to 1 day at 37 °C, 1 week at 25 °C, 1 month at 4 °C, or at –20 °C or colder temperatures for longer term storage prior to RNA preparation.

1. *Prepare tissue.* Quickly slice and weigh a piece of fresh, frozen, or RNA*later* stabilized tissue. Up to 30 mg may be used per preparation, with the exception of spleen, which requires no more than 20 mg. Transfer to an appropriate vessel for homogenization.
   Note: It is essential to disrupt fresh tissue in the RNA Lysis Solution/2-ME mixture as quickly as possible to minimize RNA degradation. Do not allow frozen tissue to thaw before disruption in the RNA Lysis Solution/2-ME mixture.

2. *Lyse tissue.* Add 300 µL of RNA Lysis Solution and 3 µL of 2-mercaptoethanol and homogenize immediately until no visible pieces remain.

To remove cell debris and to prevent the RNA Binding Plate from clogging it is recommended to filter the lysate through the Filter Plate (VM03 not included) before applying the lysate to the RNA Binding Plate. Alternatively, the homogenized tissue samples can be centrifuged for 5 minutes at maximum *g*-forces and the supernatant transferred to a suitable plate. Once this is complete, proceed to the standard protocol and add RNA Wash Buffer 3.

RNA Isolation using vacuum manifold

1. *Prepare binding*
   Depending on the volume of RNA Lysis Solution used in the lysis step add **300 µL or 130 µL** of **RNA Wash Buffer 3** (for lysates from cells grown in 96 well plates), to each well of the lysis plate or tube. Mix by pipetting up and down at least 10-15 times.
   Note: RNA Lysis Solution and RNA Wash Buffer 3 have to be used in the same volume ratio.

2. *Prepare the GenElute Vaccum Manifold*.
   a. The spacers are inserted notch side up into the grooves located on the short sides of the manifold.
   b. The waste container is placed into the manifold base.
   c. Insert Binding Plate into the manifold lid.
   d. Close the vacuum manifold's valve, check and adjust the vacuum pressure (pressure difference -200 mbar).

3. *Transfer crude lysates to RNA Binding Plate*. Place a RNA Binding Plate into vacuum manifold's lid and apply the samples to the wells.

4. *Bind RNA to silica membrane*. Apply vacuum until all lysates have passed through the wells (-0.2 bar; 1 minute). Release the vacuum.

5. *Desalt the silica membrane.* Desalt the membrane by adding 500 µL of RNA Wash Buffer 2 to each well and apply vacuum (-0.2 bar, 3 minutes) until all buffer has passed through the wells. Release the vacuum.

6. *DNase incubation.* Pipette 95 µL of the prepared rDNase reaction mixture (from step 2 of the preparation instructions) directly to the bottom of each well of the RNA Binding Plate and incubate at room temperature for 15 minutes. Do not touch the silica membrane with the pipette tips.
   Note: Be sure all of the rDNase reaction mixture gets into contact with the silica membrane, and the membrane is completely wetted.

7. *Wash silica membrane*. Add 500 µL of RNA Wash Buffer 1 to each well of the RNA Binding Plate. Apply vacuum (-0.2 bar; 1 minute) until all Wash Buffer 1 has passed through the wells. Release the vacuum.

   Add 800 µL of RNA Wash Buffer 2 to each well of the RNA Binding Plate. Apply vacuum (-0.2 bar; 1 minute) until all Wash Buffer 2 has passed through the wells. Release the vacuum.

   Add 500 µL of RNA Wash Buffer 3 to each well of the RNA Binding Plate. Apply vacuum (-0.2 bar; 1 minute) until all Wash Buffer 3 has passed through the wells.

8. *Remove Wash Plate*. After the final wash, close the valve, release the vacuum, and remove the RNA Binding Plate. Put the plate on a clean paper towel to remove residual wash buffer which contains ethanol. Remove the manifold lid, Wash Plate, and waste container from the GenElute Vacuum Manifold.

9. *Dry Binding Plate*. Remove any residual Wash Solution from the RNA Binding Plate. If necessary, tap the outlets of the RNA Binding Plate on clean paper sheets (supplied with the Wash Plate) or soft tissue until no drops come out. Insert the RNA Binding Plate into the lid and close the manifold. Apply vacuum of (-0.6 bar) for at least 10 minutes to dry the membrane completely to eliminate any traces of ethanol.
   Note: The ethanol in RNA Wash Buffer 3 inhibits enzymatic reactions and has to be removed completely before RNA elution.

10. *Elute highly pure total RNA*. Insert the Elution Plate onto the spacers inside the manifold base. If eluting into microwell plates, spacers should be inserted into the manifold base before inserting the microwell plate. Pipette 75 µL of RNase-free water directly to the bottom of each well. Incubate for 2 minutes at room temperature. Build up the vacuum with the valve closed. Once the maximum vacuum (- 0.5 bar) is achieved, open the valve and apply vacuum for 1 minute.

    Alternatively, elution in standard PCR plates is possible. Elution into PCR plates can be performed by placing a PCR plate onto an Square-well Block resting on the spacers "Square-well Block" in the manifold.

RNA Isolation using a centrifuge

1. *Prepare binding*.
   Depending on the volume of RNA Lysis Solution used in the lysis step add **300 µL or 130 µL** of **RNA Wash Buffer 3** (for lysates from cells grown in 96 well plates), to each well of the lysis plate or tube. Mix by pipetting up and down at least 10-15 times.
   Note: RNA Lysis Solution and RNA Wash Buffer 3 have to be used in the same volume ratio.

2. *Transfer crude lysates to RNA Binding Plate.* Place a RNA Binding Plate on a Square-well Block and transfer lysates to the wells of the RNA Binding Plate.

3. *Bind RNA to silica membrane.* Centrifuge for 2 minutes at 5,600-6,000 × *g*. Empty Square-well Block.

4 *Desalt silica membrane.* Desalt the membrane by adding 500 µL of RNA Wash Buffer 2 to each well and centrifuge for 2 minutes at 5,600-6,000 × *g*. Empty Square-well Block.

5. *DNase incubation.* Pipette 95 µL of the prepared rDNase reaction mixture (from preparation instructions, step 2) directly to the bottom of each well of the RNA Binding Plate and incubate at room temperature for 15 minutes. Do not touch the silica membrane with the pipette tips
   Note: Be sure that all of the rDNase reaction mixture gets into contact with the silica membrane, and the membrane is completely wetted.

6. *Wash silica membrane*. Add 500 µL of RNA Wash Buffer 1 to each well of the RNA Binding Plate. Place the RNA Binding Plate on the Square-well Block into the rotor bucket and centrifuge for 2 minutes at 5,600-6,000 × *g*. Empty Square-well Block.

   Add 800 µL of RNA Wash Buffer 2 to each well of the RNA Binding Plate and centrifuge for 2 minutes at 5,600-6,000 × *g*. Empty Square-well Block.

   Add 500 µL of RNA Wash Buffer 3 to each well of the RNA Binding Plate and centrifuge for 10 minutes at 5,600-6,000 × *g*. Empty Square-well Block.

7. *Dry Binding Plate.*
   Residual wash buffer from the RNA Binding Plate is removed by the prolonged centrifugation time of 10 minutes after adding RNA Wash Buffer 3 as described in the previous step. This prolonged time is necessary to eliminate traces of ethanol.
   Note: The ethanol in RNA Wash Buffer 3 inhibits enzymatic reactions and has to be removed completely before RNA elution.

8. *Elute highly pure total RNA*. For elution using centrifuge, place the RNA Binding Plate onto a Round-well Block (included in the kit) and pipette 75 µL of RNase-free water directly to the bottom of each well. Make sure all of the water contacts the silica membrane and that the membrane is completely wetted. Incubate for 2 minutes at room temperature and spin for 3 minutes at 5,600-6,000 × *g*.

   Alternatively, elution in an Square-well Block (or standard PCR plate) is possible. For elution, place the RNA Binding Plate on top of a Square-well Block and centrifuge. Elution into PCR plates can be performed by placing a PCR plate between the RNA Binding Plate and Square-well Block before centrifugation.

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Troubleshooting Guide

Problem	Cause	Solution
RNA is degraded or no RNA obtained	Sample material	Sample material not fresh. Whenever possible, use fresh material
	RNase contamination	Create an RNase-free environment on the worktable. Clean trough reservoirs with appropriate solutions. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended.
		Do not return unused buffer from the trough reservoir to the bottle.
		Use sterile tips with filter.
		Add 1 % 2-mercaptoethanol to RNA Lysis Solution.
Poor RNA quality of yield	Reagents not applied or prepared properly	Reagents not properly prepared. Add the indicated volume of RNase-free water to the DNase vial and 96-100% ethanol to RNA Wash Buffer 2, Concentrate and RNA Wash Buffer 3, Concentrate and mix.
		Add 1 % 2-mercaptoethanol to RNA Lysis Solution.
	Kit Storage	Store aliquots of the reconstituted DNase at −18 °C
		Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation.
		Keep bottles tightly closed in order to prevent evaporation or contamination.
	Sample material	If using more than 10^6 cells, use a shaker or a commercial homogenizer for optimal homogenization of the starting material.
	Elution	Be sure that all of the water gets into contact with the silica membrane. No water drops should stick to the walls of the columns. The membrane has to be wetted completely.
	Clogging of the RNA Binding Plate	If using too much sample or if tissue lysate has not been successfully cleared using the RNA Filter Plate, clogging of the RNA Binding Plate may appear. To prevent this reduce sample amount and raise time for vacuum filtration or centrifugation steps. If clogging happens during the run, take the remaining lysate off the RNA Binding Plate, discard it, and proceed with the desalting step.
Contamination of RNA with genomic DNA	DNase not active	Reconstitute and store lyophilized DNase according to instructions in preparation instructions
	Too much material used	Reduce quantity of tissue used
		Increase mixing cycles after adding RNA Wash Buffer 3 to the lysate
Suboptimal performance of RNA in downstream experiments	Carryover of ethanol	Be sure to remove all of the ethanolic RNA Wash Buffer 3 after the final washing step. Dry the RNA Binding Plate for at least 10 minutes with maximum vacuum or by 10 minutes centrifugation
Vacuum manifold	Vacuum pressure is not sufficient	Check if the vacuum manifold lid fits tightly on the manifold base if vacuum is turned on.
Buffers	Buffer volumes are not enough	Buffers are delivered in sufficient, but limited amounts. Calculate the required buffer volumes and pour an additional amount of 10% into the reservoirs.
		Do not return unused buffer from reservoir to the bottle to avoid contaminations.