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Product Information

GenElute™ Single Cell RNA Purification Kit

Catalog number **RNB300**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

GenElute™ Single Cell RNA Purification Kit provides a rapid and sensitive method for the isolation and purification of total RNA from small input amounts of cultured animal cells, sorted cells, and micro dissected samples including laser-capture micro dissection (LCM). The kit can recover RNA from as little as a single cell to 2×10^5 cells. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Purification is based on spin column chromatography using a proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first lysing the cells with the provided Buffer RL (please see the flow chart on page 4). Ethanol is then added to the lysate, and the solution is loaded onto a Single Cell RNA Spin Column. The resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the column, while the contaminating proteins will be removed in the flowthrough or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution A in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Solution A. The special design of the micro spin-column allows a small elution volume of as little as 8 µL. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Components

Materials Provided	50 preps
Buffer RL	40 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Single Cell RNA Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Buffer RL	40 mL
Wash Solution A	38 mL
Elution Solution A	6 mL

Reagents and Equipment Required But Not Provided

You must have the following in order to use the Single Cell RNA Purification Kit:

For All Protocols

- Benchtop microcentrifuge
- 96-100 % ethanol
- β-mercaptoethanol (optional)

For Single Cell Protocol

- 96-well collection plate for cell collection

For Animal Cell Protocol

- PBS (RNase-free)

For Laser-Captured Micro dissection (LCM) Protocol

- Sterile fine forceps
- Water bath or heat block set at 42 °C
- 70% ethanol

Precautions and Disclaimer
For Research Use Only. Not for use in diagnostic procedures.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

The Buffer RL contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

β-mercaptoethanol is suggested to be added to Buffer RL; β-mercaptoethanol is toxic and should be dispensed in a fume hood.

Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.

RNases are very stable and robust enzymes that degrade RNA. The first step when preparing to work with RNA is to create an RNase-free environment. The RNA area should be located away from microbiological work stations. Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only. All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water. Clean all surfaces with commercially available RNase decontamination solutions. When working with purified RNA samples, ensure that they remain on ice during downstream applications. Care must be taken not to introduce RNAse especially during the final wash and elution.

Reagents to be prepared
Before beginning the procedure, prepare the following:

1. Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to the supplied bottle(s) containing the concentrated Wash Solution A. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
2. The use of β-mercaptoethanol in lysis is highly recommended for most animal tissues, particularly those known to have a high RNAse content (example: pancreas), as well as for most plant tissues, and nasal and throat swabs. It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of Buffer RL required. β-mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the Buffer RL can be used as provided. It is important to work quickly during this procedure.

Storage/Stability
All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

Procedure
Note: All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

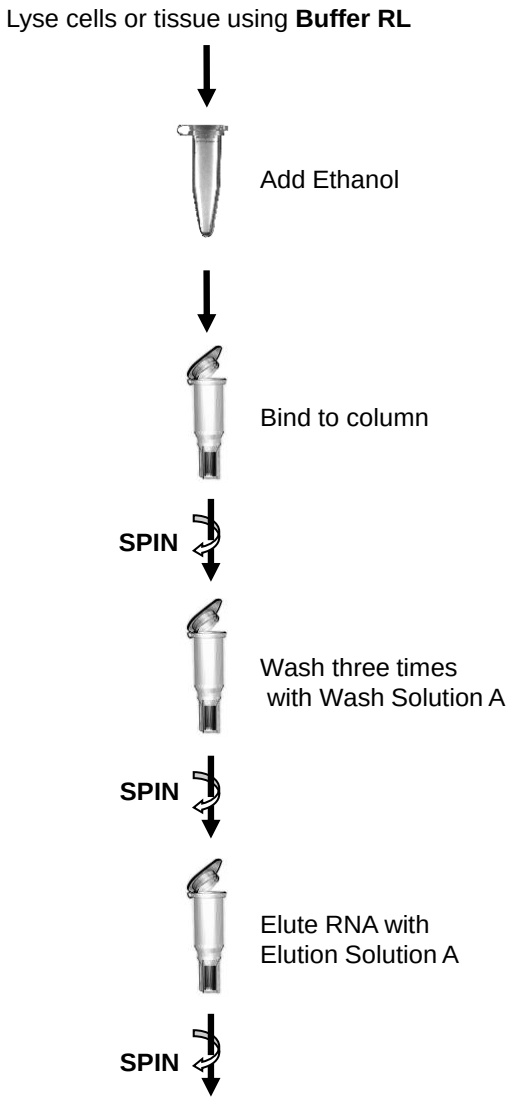
where *RCF* = required gravitational acceleration (relative centrifugal force in units of *g*); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

There are primarily 2 sections to the procedure of RNA Total extraction using GenElute™ Single Cell RNA Purification Kit:

- Preparation of Lysate
- Total RNA Purification

Flowchart

Procedure for Purifying Total RNA using GenElute™ Single Cell RNA Purification Kit



Section 1:

Preparation of Lysate from Various Cell Types

Sample types applicable:

- Single animal Cell
- Cultured Animal cells
- Laser-Captured Microdissection (LCM)

Note: Ensure that all solutions are at room temperature prior to use.

1A. Lysate Preparation from Single Animal Cell

Notes:

- Freshly sorted cells are recommended for this protocol.
- Cells can be sorted by various procedures including classic flow cytometry and fluorescence-activated cell sorting (FACS)
- Ensure that all the components of the flow cytometer (such as dip tube, septa, flow cell, all tubing lines, and nozzles) are decontaminated for RNases using an appropriate reagent

Procedure

1A (i). Cell Lysate Preparation from from Single Cell or Sorted Cells

a. Aliquot 100 µL of **Buffer RL** to an RNase-free microcentrifuge tube or into each well of a 96 well collection plate.
b. Sort the cells directly into the aliquoted **Buffer RL**. Mix by pipetting up and down a few times. If the cells are collected onto a 96 well plate, transfer the lysate to an RNase-free microcentrifuge tube.
c. Add 100 µL of 70% ethanol (provided by the user) to the lysate. Mix by vortexing.

Proceed to Binding RNA to Column step.

1B. Lysate Preparation from Cultured Animal Cells

- The maximum recommended input of cells is 2×10^5. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, each well of a confluent 96 well plate of HeLa cells will contain $2\text{-}5 \times 10^4$ cells.
- Cell pellets can be stored at –70 °C for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- Frozen cell pellets should not be thawed prior to beginning the protocol. Add the Buffer RL directly to the frozen cell pellet **(Step 1B(ii)c).**

Proceed to Binding RNA to Column step

Procedure

1B(i). Cell Lysate Preparation from Cells Growing in a Monolayer

a. Aspirate medium and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
b. Add 200 µL of **Buffer RL** directly to culture plate.
c. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
d. Transfer lysate to a microcentrifuge tube.
e. Add 120 µL of 96–100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Proceed to Binding RNA to Column step.

1B (ii). Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

a. Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than 200 × *g* (~2,000 rpm) for 10 minutes to pellet cells.
b. Carefully decant the supernatant.

Note: For inputs of over 10^5 cells, 5-10 µL of medium may be left behind with the pellet in order to ensure the pellet is not dislodged. For inputs of fewer than 10^5 cells, 30-50 µL of medium may be left behind in order to ensure that the pellet, which could be invisible, is not dislodged.

c. Add 350 µL of **Buffer RL** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure the entire pellet is completely dissolved before proceeding to the next step.
d. Add 200 µL of 96–100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Proceed to Binding RNA to Column step.

1C. Lysate Preparation from Laser-Captured Microdissection (LCM)

Notes: LCM samples obtained from frozen sections are recommended. Formalin-fixed, paraffin-embedded sections may also be used. However, RNA isolated from FFPE samples generally has poorer quality than that from frozen sections.

Procedure

a. Aliquot 300 µL of **Buffer RL** to an RNase-free microcentrifuge tube.
b. Remove the thermoplastic film containing the captured cells using sterile fine forceps. Carefully submerge the sample into the aliquoted **Buffer RL**. Close the microcentrifuge cap.
c. Incubate the sample at 42 °C for 30 minutes. Apply vortex for 15 seconds after every 10 minutes.
d. At the end of the incubation, vortex the tube one more time for 15 seconds. The thermoplastic film may be removed at this point using sterile fine forceps. Otherwise, proceed to **Step 1Ce.**
e. Add 300 µL of 70% ethanol (provided by the user) to the lysate. Vortex to mix.

Proceed to Binding RNA to Column step.

Section 2.

Binding RNA to Column

Note: The remaining steps of the procedure for the purification of total RNA are the same from this point forward for all the different types of lysate.

a. Assemble a Single Cell RNA Spin Column with one of the provided collection tubes.
b. Apply up to 600 µL of the lysate with the ethanol (from **Step 1**) onto the column and centrifuge for 1 minute at **3,500 × *g* (~6,000 rpm)**.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 × *g* (~14,000 rpm)**.

c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Depending on your lysate volume, repeat Step **2b** and **2c** as necessary.

Optional Step:
GenElute Single Cell RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that a RNase-Free DNase I Kit be used for this step. This step should be performed at this point in the protocol.

Section 3.
Column Wash

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute at **14,000 × *g* (~14,000 rpm)**.

Note: Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps **3a** and **3b** to wash column a second time.
d. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute at **14,000 × *g* (~14,000 rpm)**.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes at **14,000 × *g* (~14,000 rpm)**in order to thoroughly dry the resin. Discard the collection tube.

Section 4.
RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 8-20 µL of **Elution Solution A** to the column.

Note: For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 8 µL is recommended

c. Centrifuge for 1 minutes at **200 × *g* (~2,000 rpm)**, followed by 1 minute at **14,000 × *g* (~14,000 rpm)** Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 × *g* (~14,000 rpm) for 1 additional minute.

Section 5.
Storage of RNA
The purified RNA sample may be stored at –20 °C for a few days. It is recommended that samples be placed at –70 °C for long term storage.

Section 6.
Quantification of RNA
For cell inputs of $\geq 1 \times 10^5$ cells, it is possible to quantify RNA using a nanospectrophotometer (such as Thermo Scientific's NanoDrop). For cell inputs between 10^2 and 10^5 cells, RNA quantification requires a highly sensitive fluorescence-based system (such as Life Technologies' RiboGreen® RNA Assay kit). For cell inputs between a single cell and 10^4 cells, quantification could be performed using RT-qPCR of an RNA transcript of high abundance (such as GAPDH or 5S rRNA) with a standard curve generated with total RNA of known concentration.

Protocol for Optional On-Column DNA Removal
GenElute Single Cell RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that a RNase-Free DNase I Kit be used for this step.

1. For every on-column reaction to be performed, prepare a mix of 15 µL of **DNase I** and 100 µL of **Enzyme Incubation Buffer** using RNase-Free DNase I Kit. Mix gently by inverting the tube a few times. **DO NOT VORTEX**.

Note: If using an alternative DNase I, prepare a working stock of 0.25 Kunitz unit/µL RNase-free DNase I solution according to the manufacturer's instructions. A 100 µL aliquot is required for each column to be treated.

2. Perform the appropriate Total RNA Isolation Procedure for your starting material up to and including "**Binding to Column**" (Steps 1 and 2 of all protocols)
3. Apply 400 µL of **Wash Solution A** to the micro spin column and centrifuge for 2 minute. Discard the flowthrough. Reassemble the spin column with its collection tube.
4. Apply 100 µL of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 14,000 × *g* (~14,000 rpm) for 1 minute.

Note: Ensure the entire DNase I solution passes through the column. If needed, spin at 14,000 × *g* (~14,000 rpm) for an additional minute.

5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.

Note: Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

6. Incubate the column assembly at 25–30 °C for 15 minutes.
7. Without any further centrifugation, proceed directly to the second wash step in the "**Column Wash**" section (Step 3c).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure the appropriate amount of Buffer RL was used for the amount of cells or tissue.
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also "Clogged Column" below.
	An alternative elution solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use.
	Low RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA content of your starting material.
	Cell Culture: Cell monolayer was not washed with PBS	Ensure the cell monolayer is washed with the appropriate amount of PBS in order to remove residual medium from cells.
	LCM: Sample was not incubated at 42 °C for 30 minutes	Ensure the incubation at 42 °C for the removal and lysis of cells from the thermoplastic film.
Clogged Column	Centrifuge temperature too low	Ensure the centrifuge remains at room temperature throughout the procedure. Temperatures below 15 °C may cause precipitates to form that can cause the columns to clog.

Problem	Possible Cause	Solution and Explanation
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "*Working with RNA"* at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20 °C for a few days. It is recommended that samples be stored at –70 °C for longer term storage.
	Frozen tissues or cell pellets were allowed to thaw prior to RNA isolation	Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
	Starting material may have a high RNase content	For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Buffer RL.
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNase I digestion on the RNA sample after elution to remove genomic DNA contamination. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

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