3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Gen Elute™ Water RNA/DNA Purification Kit, 0.45 µm
Catalog number RDP100

TECHNICAL BULLETIN

Product Description
Sigma's Gen Elute Water RNA/DNA Purification Kit provides a convenient and rapid method for the detection of microorganisms from environmental water samples. The kit allows for the rapid isolation and purification of total RNA and DNA simultaneously from the microorganisms found in small and large samples of water. The total RNA and DNA (including genomic DNA) are isolated from all the microorganisms found in the water, including bacteria, fungi and algae without the use of any inhibitory organic substances. The water sample is first passed through a 0.45 µm filter, and the microorganisms present in the water are captured. Both the RNA and DNA are then column purified in under 45 minutes using a single column. The purified RNA and DNA are highly concentrated, and can be used directly in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, Southern blotting and sequencing reactions.

Purification is based on spin column chromatography using proprietary resin as the separation matrix. The process involves first collecting the microorganisms present in the water sample using the provided 0.45 µm Filter Column. The water is passed through the column using a vacuum apparatus, and the filter containing the microorganisms is then removed and transferred into a provided Bead Tube. Lysis Buffer E is then added to the Bead Tube, and the tube is vortexed for 5 minutes followed by a 10 minute incubation at 65 °C. After incubation the lysate is collected, ethanol is added and the solution is loaded onto a spin-column. The resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA and DNA will bind to the column while the proteins are removed in the flow through. Next, an optional step can be carried out in which the genomic DNA can be digested allowing for a more pure RNA sample to be isolated. Alternatively, the RNA can be digested resulting in a more pure genomic DNA sample. The bound nucleic acid is then washed twice with the provided Wash Solution A in order to remove any impurities and the purified RNA and/or DNA is eluted with the Elution Buffer H. The kit purifies genomic DNA, and all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA).

Components

Component	50 preps
Lysis Buffer E	15 mL
Wash Solution A	18 mL
Enzyme Incubation Buffer B	6 mL
Elution Buffer H	6 mL
Mini Spin Columns	25
Filter Columns (0.45 µm)	25
Bead Tubes	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
Product Insert	1

Reagents and Equipment Required But Not Provided
You must have the following in order to use the Water RNA/DNA Purification Kit:

- Vacuum apparatus
- Forceps
- Flat bed vortex or bead beater equipment
- Benchtop microcentrifuge
- RNase-free microcentrifuge tubes
- 96-100% ethanol
- 70% ethanol
- Water bath or incubator heated to 65 °C

Precautions and Disclaimer
This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Safety Data Sheets (SDSs).

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes.

The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- 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. It is recommended that gloves are changed frequently to avoid contamination
- 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

Reagents to be prepared

Before beginning the procedure, prepare the following:

1) Prepare a working concentration of the **Wash Solution A** by adding 42 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

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

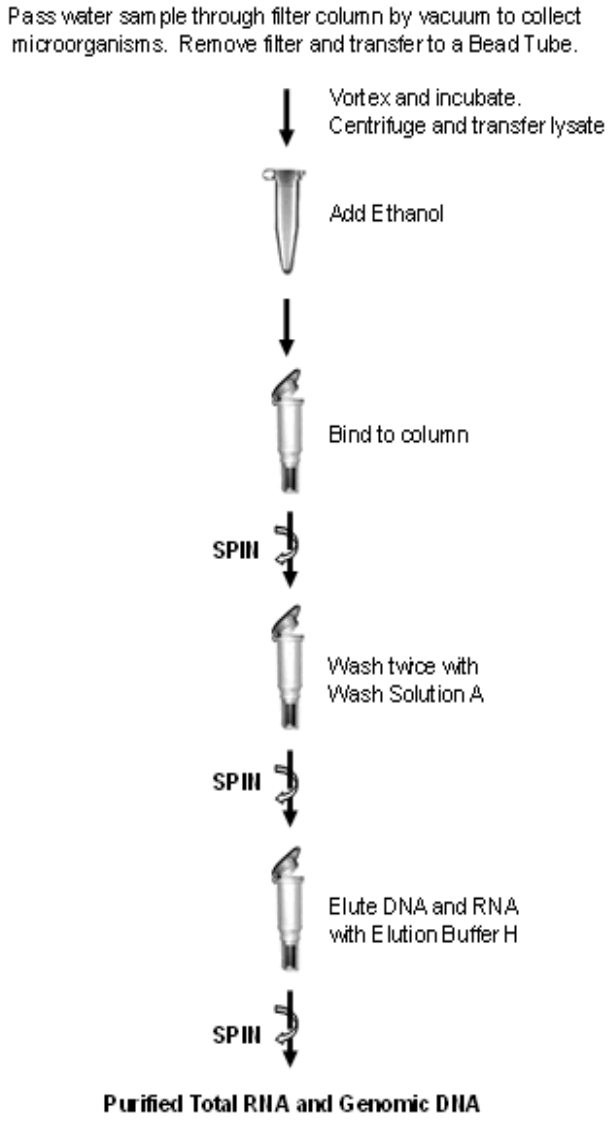
Note: All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for the different microcentrifuge steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature.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

Flowchart

Procedure for Purifying Genomic DNA and Total RNA using Water RNA/DNA Purification Kit



Procedure

1. Lysate Preparation using a Vacuum Apparatus

a. Obtain a water sample that is 10 mL–100 mL, depending on the water source (see Notes Prior to Use above).
b. Place the provided Filter Column onto a vacuum apparatus.
c. Transfer up to 20 mL of water to the filter and allow the total volume to pass. This may be repeated as required in order to allow the entire water sample (up to 100 mL) to pass through the filter.
d. Cut the filter out by running a sterile scalpel blade along with edge of O-ring, located on top of the filter. Alternatively, remove the O-ring from the filter column by using tissue forceps which have 1x2 teeth.
e. Carefully remove the filter from the filter column using sterile forceps, and transfer to a provided Bead Tube.

Note: Remove the filter by picking it up by the edges/corner. Avoid touching the center of the filter. Ensure when placing the filter into the Bead Tube that the upper surface of the filter is facing the center of the tube.

f. Add 500 µL of **Lysis Buffer E** to the Bead Tube and secure the tube horizontally on a flat-bed vortex pad with tape, or in any commercially available bead beater equipment. Vortex for 5 minutes at maximum speed.

Note: The appearance of some white foam during the homogenization is common. This is due to detergents present in the Lysis Buffer E and will not affect the protocol.

g. Incubate at 65 °C for 10 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube. Ensure that the filter does not become dry.
h. Centrifuge the tube for 1 minute at **14000 × g (~14,000 RPM)**.
i. Transfer the lysate to another RNase-free microcentrifuge tube (provided by the user). Note the volume.
j. Add an equal volume of 70% ethanol (provided by the user) to the lysate collected above (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **Proceed to Step 2.**

2. Binding Nucleic Acids to Column

a. Assemble a spin column with one of the provided collection tubes.
b. Apply up to 600 µL of the lysate with ethanol onto the column and centrifuge for 1 minute at **14,000 x g (~14,000 RPM)**. Discard the flow through and reassemble the spin column with the collection tube

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.

c. Depending on your lysate volume, repeat step **2b** if necessary.

3. DNase Treatment (Optional)

This optional step is carried out if genomic DNA-free RNA is required. It is recommended that RNase-Free DNase Kit be used for this step.

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 2 minutes. Discard the flowthrough.

Note: Ensure the entire wash solution 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. Apply 100 µL of **Enzyme Incubation Buffer B** containing 15 µL RNase-free DNase I to the column. If using an alternate DNase I, apply 100 µL of **Enzyme Incubation Buffer B** containing 25 units of DNase I to the column. **Note:** If you wish to isolate RNA-free genomic DNA, apply 100 µL of **Enzyme Incubation Buffer B** containing 10 units of RNase A (user provided) to the column and proceed as written below.
c. Centrifuge for 1 minute at 14,000 x g (~14 000 RPM). Ensure that the entire DNase I solution passes through the column. Repeat the step if needed.
d. After centrifugation, pipette the flowthrough that is present in the collection tube back onto the top of the column.

Note: Ensure Step 3d is performed in order to ensure maximum DNAse activity and to obtain maximum yields of RNA.

e. Incubate the whole unit at room temperature for 15 minutes.
f. Proceed to Step **4c** (second Column Wash) without further centrifugation.

4. Column Wash

a. Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute.

Note: Ensure the entire wash solution 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 flow through and reassemble the column with its collection tube.
c. Repeat step **4a** to wash column a second time.
d. Discard the flow through and reassemble the spin column with its collection tube.
e. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. Nucleic Acid Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL of **Elution Buffer H** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x *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 x g (~14,000 RPM) for 1 additional minute.
d. **(Optional):** An additional elution may be performed if desired by repeating steps **4b** and **4c** using 50 µL of Elution Buffer. The total yield can be improved by an additional 20–30% when this second elution is performed.

6. Storage of DNA and RNA

The purified nucleic acids may be stored at –20 °C for a few days. It is recommended that samples be placed at –70 °C for long term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Clogged Filter Column	Input volume of water sample was too high	The amount of sample input may need to be decreased to prevent clogging of the Filter Column, particularly for turbid water samples.
	Pre-filtering is necessary	For highly turbid water samples or samples containing high level of sediments, pre-filtration of the sample might be necessary. Pass the sample through a 1-8 μm filter prior to applying to the Filter Column (0.45 μm) to remove debris.
Poor DNA/RNA Recovery	Lysis was not completed	Ensure the filter is has not dried out during the 65 °C incubation step. Alternatively, increase the incubation time at 65 °C to 15 minutes.
	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	Ensure that 42 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use.
	An alternative elution buffer was used	It is recommended that the Elution Buffer H supplied with this kit be used for maximum DNA recovery.
DNA/RNA does not perform well in downstream applications	Ethanol carryover	Ensure that 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.
	DNA was not washed twice with the provided Wash Solutions A	Traces of salt from the binding step may remain in the sample if the column is not washed twice with the Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	PCR reaction conditions is need to be optimized	Take steps to optimize the PCR conditions being used, including varying the amount of template, changing the source of *Taq* polymerase, looking into the primer design and adjusting the annealing conditions.

GenElute is a trademark of Sigma-Aldrich Co. LLC

JG,RC,PHC 09/15-1