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Water RNA/DNA Purification Kit — Product Insert

Product # 26480

Norgen's 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 kit provides bead tubes that can be used to homogenize microorganisms on a filter (not provided), and then both the RNA and DNA are 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.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The process involves the homogenization of a water filter (not provided) with Lysis Buffer E by vortexing 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. Norgen's 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 flowthrough. 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).

Specifications

Kit Specifications	
Maximum Spin Column Loading Volume	650 µL
Elution Volume	100 µL
Time to Complete 10 Purifications	45 minutes

Kit Components

Component	Product # 26480 (50 preps)
Lysis Buffer E	2 x 15 mL
Wash Solution A	38 mL
Enzyme Incubation Buffer B	6 mL
Elution Buffer H	6 mL
Mini Spin Columns	50
Bead Tubes	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Advantages

- Fast and easy processing using a rapid spin-column format
- No phenol or chloroform extractions
- Isolate high quality genomic DNA and total RNA from large and small water samples
- No extra ethanol precipitation is required for DNA or RNA concentration

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

Precautions and Disclaimers

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 Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Water RNA/DNA Purification Kit:

- 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^{o}C

Working with RNA

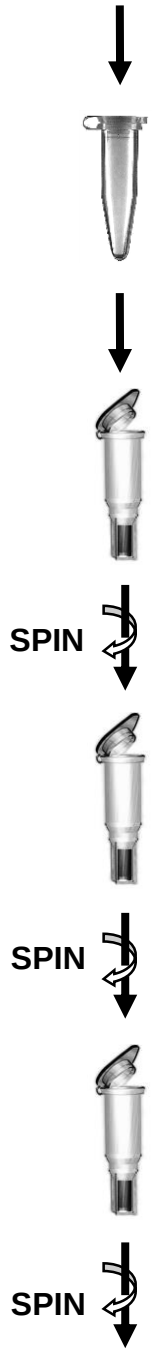
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense 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

Flow Chart

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

Remove filter from a water filter device and transfer to a Bead Tube.



Purified Total RNA and Genomic DNA

Procedures

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.

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 20,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed microcentrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- 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 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.
- Pre-heat a water bath or an incubator to 65°C
- It is important to work quickly when purifying RNA.

1. Lysate Preparation

a. The starting material for this procedure is a filter device used for water collection and filtration. Carefully remove the filter from the filter device using sterile forceps and transfer to a provided Bead Tube. If the diameter of the filter is bigger than 0.75 inch (~19 mm), cut into several pieces with sterile scissors and transfer to the 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.

b. Add 500 µL of **Lysis Buffer E** to the Bead Tube.

c. Secure the tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g. MP Biomedicals' FastPrep®-24 Instrument). Vortex for 30 seconds at 4M/S using a FastPrep®-24 instrument, or 5 minutes using a flat-bed vortexer at maximum speed. Alternatively, optimize the time and speed according to the manufacturer's manual.

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.

d. 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.

e. Centrifuge the tube for 1 minute at **20,000 × g (~14,000 RPM)**.

f. Transfer the lysate to another RNase-free microcentrifuge tube (provided by the user). Note the volume.

g. 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 **20,000 x g (~14,000 RPM)**. Discard the flowthrough 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 Norgen's RNase-Free DNase I Kit (Product # 25710) 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 of Norgen's RNase-free DNase I (Product# 25710) 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 20,000 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 flowthrough and reassemble the column with its collection tube.

c. Repeat step **4a** to wash column a second time.

d. Discard the flowthrough 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 **20,000 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
Poor DNA/RNA Recovery	Lysis was not completed	Ensure the filter is has not dried out during the 65ºC incubation step. Alternatively, increase the incubation time at 65ºC 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 90 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.
	RNA degradation	RNases are very stable and robust enzymes that degrade RNA. Please see section “Working with RNA” to prevent RNA degradation.
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/RNA 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.

Related Products	Product #
Water RNA/DNA Purification Kit (0.22 µm)	26400
Water RNA/DNA Purification Kit (0.45 µm)	26450
RNase-Free DNase I Kit	25710
Plant/Fungi DNA Isolation Kit	26200
Plant/Fungi RNA Purification Kit	25800
Soil DNA Purification Plus Kit	64000
1kb RNA Ladder	15003
UltraRanger 1kb DNA Ladder	12100

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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