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Fisher BioReagents®

SurePrep™ Water RNA/DNA Purification Kit

Product Cat. # BP2814-25

Instruction Manual

I. Introduction

A. Product Description

The SurePrep™ Water RNA/DNA Purification Kit provides a convenient and rapid method for the detection of microorganisms from environmental water and wastewater 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 chemicals. 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.

B. Overview of Procedure

Purification is based on spin column chromatography using a 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 Solution 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 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 in order to remove any impurities, and the purified RNA and/or DNA is eluted with the Nucleic Acid Elution Buffer. 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).

C. Kit Specifications

Kit Specifications	
Minimum Water Input	10 mL
Maximum Water Input	100 mL
Maximum Filter Column Loading Volume	20 mL
Maximum Spin Column Loading Volume	600 μL
Elution Volume	100 μL
Time to Complete 10 Purifications	45 minutes

D. Advantages

- Rapid and convenient method to detect microorganisms in environmental water samples
- Fast and easy processing using rapid spin-column format
- No phenol or chloroform extractions
- Isolate high quality genomic DNA and total RNA from large and small water samples

E. Kit Components

Component	Catalog # BP2814-25 (25 preps)
Lysis Solution	15 mL
Wash Solution	11 mL
Enzyme Incubation Buffer	4 mL
Nucleic Acid Elution Buffer	4 mL
Spin Columns	25
Filter Columns (0.45 μm)	25
Bead Tubes	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
Product Insert	1

F. Storage Conditions and Product Stability

All kit solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 2 years in their unopened containers.

G. 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 (MSDS). The MSDS can be requested through our Customer Service Department.

H. Customer-Supplied Reagents and Equipment

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

- Vacuum apparatus
- Forceps
- Flat bed vortex or bead beater equipment
- Benchtop microcentrifuge
- RNase-free microcentrifuge tubes
- 95 - 100% ethanol
- 70% ethanol
- Water bath or incubator heated to 65^{o}C
- RNase-free DNase I (optional)
- DNase-free RNase A (optional)

I. Working with RNA

RNases are stable, robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to inactivate 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.

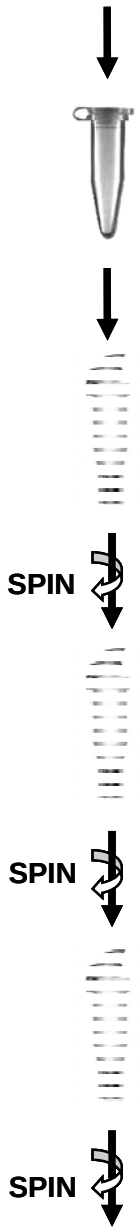
- The RNA area should be located away from microbiological work stations

- 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

Flowchart

Procedure for Isolating Genomic DNA and Total RNA Using the SurePrep Water RNA/DNA Purification Kit

Pass water sample through filter column by vacuum to collect microorganisms. Remove filter and transfer to a Bead Tube.



Purified Total RNA and Genomic DNA

II. Set-Up and Preparation of Sample Lysate

A. Equipment Preparation

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.

If you do not own a variable speed microcentrifuge consider purchasing Thermo Scientific's Sorvall Legend benchtop model that offers power, safety and convenience.

- Choice of 17,000 or 21,000 x g (for RNA purification using SurePrep kits, the microcentrifuge with 17,000 x g is sufficient)
- Holds 36 x 0.5 mL microtubes, 24 x 2 mL tubes or 8 x 8 PCR
- Unique ClickSeal™ bio-containment rotor lid for safe processing of infectious specimens
- Fast acceleration and deceleration speeds up your protocols
- Broad range of rotors supports virtually any application
- Intuitive controls and vivid display
- Highly resistant materials allow vigorous cleaning and autoclaving

Sorvall Legend Micro Centrifuges

Technical Specifications

	Sorvall Legend Micro 17 & 17R	**Sorvall Legend Micro 21 & 21R**
Max g-force:	17,000	21,100
Max RPM:	13,300	14,800
Noise level:	<55 dBA	<56 dBA
Time set range:	1 min - 99 min; 1 min increments	1 min - 99 min; 1 min increments
Temp set range:	Set from -9 °C to +40 °C; per 1 °C increment	Set from -9 °C to +40 °C per 1 °C increment

Ordering Information

	Cat. No.	Cat. No.
Sorvall Legend Micro 17/17R	230V 50/60Hz	120V 60 Hz
Sorvall Legend Micro 17, includes 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002430	75002431
Sorvall Legend Micro 17R,includes 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002440	75002441
Sorvall Legend Micro 21/21R	230V 50/60Hz	120V 60 Hz
Sorvall Legend Micro 21, incl. 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002435	75002436
Sorvall LegendMicro 21R, incl. 24 x 1.5/2.0 mL rotor with ClickSeal bio-containment lid	75002445	75002446

For detailed product specifications, information on additional rotors, lids and adapters visit **www.thermo.com**

B. Preparation of Lysate Using a Vacuum Apparatus

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~12,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge 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 nucleic acid **Wash Solution** by adding 25 mL of 95 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 36 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^{o}C.
- For optimal kit performance we recommend processing 10 mL to 100 mL of water, depending on the sample. For clear water samples, the maximum input of 100 mL can be processed. For turbid water samples we recommend processing smaller volumes, as the suspended solids or sediment may clog the provided filter.
- It is important to work quickly when purifying RNA.
- Please be advised that all RPM values referenced in the protocols next to the actual g force are calculated for Sorvall Legend Microcentrifuge with rotor for 24 x 2mL tubes.

Lysate Preparation from Filtered Water Samples

a. Obtain a water sample that is 10 to 100 mL, depending on the type of 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 0.45 µm filter.
d. Cut the filter out by running a sterile scalpel blade along the 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.g. VWR Tissue forceps Cat. No. 25607-608).
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 Solution** 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 (e.g. Scientific Industries' Disruptor Genie^{TM}). Vortex for 5 minutes at maximum speed.
g. Incubate at 65^{o}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 (~12,000 RPM)**.
i. Transfer the supernatant to another RNase-free microcentrifuge tube (not provided). 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 **III A**, Binding Nucleic Acids to Column.

III. Purifying Nucleic Acids from Water Sample Lysates

A. 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 (~12,000 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 one minute.

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

B. DNase Treatment (Optional)

This optional step is carried out if genomic DNA-free RNA is required.

a. Apply 400 μL of **Wash Solution** 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 one minute.

b. Apply 100 μL of **Enzyme Incubation Buffer** containing 25 units of RNase-free DNase I (user provided) to the column.

Note: If you wish to isolate RNA-free genomic DNA, apply 100 μL of **Enzyme Incubation Buffer** 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 (~12,000 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 **d** 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 **C** below (second Column Wash) without further centrifugation.

C. Column Wash

a. Apply 500 μL of **Wash Solution** 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 one minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Again, apply 500 μL of **Wash Solution** to the column and centrifuge for 1 minute.
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.

D. Nucleic Acid Elution

a. Place the column into a fresh 1.7 mL **Elution Tube** provided with the kit.
b. Add 50 µL of **Nucleic Acid Elution Buffer** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~1,500 RPM)**, followed by a 1 minute spin at **14,000 x *g* (~12,000 RPM).** Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~12,000 RPM) for one additional minute.
d. **(Optional):** An additional elution may be performed if desired by repeating steps **b** and **c** above using 50 µL of **Nucleic Acid Elution Buffer**. The total yield can be improved by an additional 20 – 30% when this second elution is performed.

E. Assessing RNA Yield and Quality by UV Absorbance

The concentration and purity of an RNA solution can be determined by absorbance (A) measurements at 260 and 280 nm. A_{260} measurements are quantitative for relatively pure RNA preparations in microgram quantities. A_{260} readings cannot distinguish between DNA and RNA, however the ratio of A_{260}/A_{280} can be used as an indication of RNA purity. For example, contaminating proteins have a peak absorption at 280 nm that will reduce the A_{260}/A_{280} ratio.

a. Determine RNA concentration by diluting an aliquot of the purified RNA solution (e.g. 1:50 dilution) in TE (10 mM Tris and 1 mM EDTA, pH 7.4). Measure absorbance of the diluted sample in a 1 mL cuvette using a traditional UV-VIS spectrophotometer at 260 and 280 nm. The spectrophotometer should first be zeroed with the TE used to dilute the sample.
b. An A_{260} of 1.0 is equivalent to 40 µg RNA/mL. Calculate the RNA concentration in µg/mL as follows:

A_{260} x dilution factor x 40 = µg RNA/mL

c. The ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the RNA purity with respect to contaminants that absorb in the UV range such as protein. Ratios of 1.8 to 2.1 indicate highly purified preparations of RNA. Contaminants such as protein that absorb at 280 nm will lower this ratio. However, RNA solutions with a ratio lower than 1.8 may function well in downstream applications such as RT-PCR and Northern blotting.

F. Assessing DNA Yield by UV Absorbance

Spectrophotometric measurement of the amount of UV irradiation absorbed by DNA is simple and accurate when the DNA sample is pure.

a. Determine DNA concentration by making an appropriate dilution of the purified DNA solution in TE (10 mM Tris and 1 mM EDTA, pH 7.4). Measure absorbance of the diluted sample in a 1 mL cuvette using a traditional UV-VIS spectrophotometer at 260 and 280 nm. The spectrophotometer should first be zeroed with the TE used to dilute the sample.
b. An A_{260} of 1.0 is equivalent to 50 µg double-stranded DNA per mL. Calculate the DNA concentration in µg/mL as follows:

A_{260} x dilution factor x 50 = µg DNA/mL

c. The ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the DNA

purity with respect to contaminants that absorb in the UV range such as protein. Ratios of 1.8 to 2.0 indicate highly purified preparations of DNA. Contaminants such as protein that absorb at 280 nm will lower this ratio.

G. Storage of RNA and DNA

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.

IV. 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 did not dry out during the 65ºC incubation step. Alternatively, increase the incubation time at 65ºC to 15 or 20 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 25 mL of 95 - 100% ethanol is added to the supplied **Wash Solution** prior to use.
	An alternative elution buffer was used	It is recommended that the **Nucleic Acid Elution Buffer** 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	Traces of salt from the binding step may remain in the sample if the column is not washed twice with the **Wash Solution**. Salt may interfere with downstream applications, and thus must be washed from the column.
	PCR reaction conditions 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, verifying primer design and adjusting the primer annealing conditions.

V. Related Products

A. Additional RNA Purification Kits

Catalog #	Product Description
BP2800-50	SurePrep™ TrueTotal™ RNA Purification Kit
BP2801-25	SurePrep™ Small RNA Purification Kit
BP2802-50	SurePrep™ RNA/DNA/Protein Purification Kit
BP2803-50	SurePrep™ Urine Exfoliated Cell RNA Purification Kit
BP2804-50	SurePrep™ Urine Bacterial RNA Purification Kit
BP2805-50	SurePrep™ Nuclear Or Cytoplasmic RNA Purification Kit
BP2806-50	SurePrep™ RNA/Protein Purification Kit
BP2807-50	SurePrep™ Leukocyte RNA Purification Kit
BP2809-50	SurePrep™ RNA Cleanup and Concentration Kit
BP2814-25	SurePrep™ Water RNA/DNA Purification Kit
BP2815-50	SurePrep™ Soil DNA Isolation Kit
BP2816-50	SurePrep™ FFPE RNA Isolation Kit
BP2817-50	SurePrep™ Plant/Fungi Total RNA Purification Kit

B. Other Fisher BioReagents Functionally Tested for RNA Research

BP2484-50	Water, Sterile (DEPC-treated) 50mL
BP2484-100	Water, Sterile (DEPC-treated) 100mL
BP561-1	Water, Sterile (RNA Grade) 1L
BP2483-100	EDTA 0.5 M (DEPC-treated) 100mL
BP2483-1	EDTA 0.5 M (DEPC-treated) 1L
BP2483-500	EDTA 0.5 M (DEPC-treated) 500mL
BP2810-50	RiboLadder™ 100b RNA Standard with loading buffers
BP2811-50	RiboLadder™ 1Kb RNA Standard with loading buffers
BP3224-5	Optizyme™ Ribonuclease Inhibitor (Human Placental) 10,000U
BP3224-1	Optizyme™ Ribonuclease Inhibitor (Human Placental) 2,500U
BP3225-5	Optizyme™ Ribonuclease Inhibitor (Porcine) 10,000U
BP3225-1	Optizyme™ Ribonuclease Inhibitor (Porcine) 2,500U
BP3222-5	Optizyme™ Ribonuclease Inhibitor (Recombinant) 10,000U
BP3222-1	Optizyme™ Ribonuclease Inhibitor (Recombinant) 2,500U
BP3226-1	Optizyme™ Recombinant DNase I (RNase-free) 1,000U
BP3226-2	Optizyme™ Recombinant DNase I (RNase-free) 2,000U
BP176-100	2-Mercaptoethanol 100g
BP535-1	Lysozyme, Egg White 1g
BP535-5	Lysozyme, Egg White 5g
BP535-10	Lysozyme, Egg White 10g
BP2476-100	Tris-EDTA, 1X Solution, pH 7.4 100ml
BP2476-500	Tris-EDTA, 1X Solution, pH 7.4 500ml
BP160-100	Agarose, Low EEO, Multipurpose 100g
BP1360-100	Agarose, Low Melting, <1kb RNA 100g
BP1356-100	Agarose, Broad Separation Range for RNA 100g
BP1302-10	Ethidium Bromide, 1% Solution 10ml
BP2900-500	MOPS 10X Solution 500ml
BP2900-1	MOPS 10X Solution 1L

Fisher BioReagents Technical Support

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