GB's Pure Spin
Corona Virus (Covid-19)RNA ISOLATION KIT
Spin Column Based

GENUINE BIOSYSTEM

KIT NAME	KIT SIZE	CAT. NO
GB's Pure Spin -Covid-19 viral RNA Isolation Kit	100T	RSCOV01100T
GB's Pure Spin-Covid-19 viral RNA Isolation Kit	250T	RSCOV01250T

PRINCIPLE

The GB's Pure Spin viral RNA Isolation kit is designed for the isolation of RNA from plasma (treated with anticoagulants other than heparin), serum, other cell-free body fluids and nasal/throat swabs collected in viral transfer medium (VTM). This kit provides reagents and spin column for isolation of viral RNA from the samples. The spin column based Viral RNA isolation kit procedures employed the glass-fiber membrane technology for the fastest and the most convenient of high purity RNA isolation, instead of conventional alcohol precipitation or phenol/chloroform extraction. Viral RNA isolation kit buffer system provides the effective binding condition of RNA to glass-fiber membrane and the impurities on the membrane are washed away by two different wash buffers. Finally, highly pure RNA is eluted with low-salt elution buffer (Ex: TE Buffer) or nuclease free water. The whole procedure takes less than 15 minutes and the purified RNA is directly used for PCR, RT-PCR, or any downstream application. The Gb's Pure Spin viral RNA Isolation kit can be used either manually or automated on standard liquid handling instruments.

KIT SPECIFICATIONS

Gb's Pure Spin viral RNA Isolation Kit is designed for rapid manual and automated small-scale preparation of viral RNA from Plasma, Serum or other body fluids and nasal/throat swabs collected in VTM. The spin column allows a high loading capacity by simultaneously small elution volume to enrich viral RNAs. The prepared viral RNA is suitable for applications like PCR, RT-PCR, or any kind of enzymatic reaction. We highly recommend using internal (low-copy) standards as well as positive and negative controls to monitor the purification, amplification, and detection processes.

KIT CONTENTS

Kit contents	For 100 Preparation	For 250 Preparation
Mini Spin Columns	100	250
Collection tubes	100	250
Lysis Buffer	10ml	25ml
Wash Buffer-1	21.5ml	54ml
Wash Buffer-2	15ml	37.5ml
Elusion Buffer	10ml	25ml
Carrier RNA	1 vial	1vial

MATERIAL TO BE SUPPLIED BY USER

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety datasheets (MSDS), available from the product supplier.

- 100% Ethanol
- Micropipettes (variable range)
- Micropippette tips
- Microcentrifuge tubes
- Vortex Mixer

STORAGE

All components of Gb's Pure Spin Viral RNA isolation kit should be stored at room temperature (15~25°C). After reconstitution of carrier RNA with Elution buffer, it should be stored in aliquots at -20°C for conservation of activity or immediately used for experiments. Under cool ambient condition, a precipitate can be formed in lysis buffer. In such a case, heat the bottle above 37°C to dissolve completely. Gb's Pure Spin Viral RNA isolation kit is guaranteed until the expiration date printed on the product label.

INTENDED TO USE

Gb's Pure Spin viral RNA Isolation Kit is intended for molecular biology applications. All due care and attention should be exercised in the handling of the products. We recommend all users of GB's products to adhere to the NIH guidelines or other standard guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

IMPORTANT INSTRUCTION

1. Read the entire procedure carefully before starting the experiment.
2. Use fresh tip while adding different solution to the TUBE.

1. PREPARATION OF SAMPLE MATERIALS

This protocol is for the purification of RNA from the COVID-19 virus collected from nasal/ pharyngeal swabs collected in 2.5 mL of Viral Transfer Media (VTM).

2. PREPARATION OF BUFFERS

Wash Buffer-1 (Concentrated): Wash Buffer-1 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle and in Table. Wash Buffer-1 is stable for 1 year when stored closed at room temperature (15–25°C), but only until the kit expiration date.

Volume of Ethanol to be added	For 100 Preparation	For 250 Preparation
Wash buffer 1	21.5ml	54ml
Ethanol	28.5ml	71ml
Final volume	50ml	125ml

Wash Buffer-2 (Concentrated): Wash Buffer-2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle and in Table 3. Wash Buffer-1 is stable for 1 year when stored closed at room temperature (15–25°C), but only until the kit expiration date.

Volume of Ethanol to be Added	For 100 Preparation	For 250 Preparation
Wash Buffer 2	15ml	37.5ml
Ethanol	35ml	87.5ml
Final Volume	50ml	125 ml

Carrier RNA: This kit is provided with carrier RNA, which can be added at lysis step if required. Carrier RNA enhances binding of nucleic acid to the spin column membrane, especially if there are very few target molecules in the sample. Before first use of the kit, add the following required volume of elution buffer to the Carrier RNA vial and mix well. Store dissolved Carrier RNA solution in aliquots at -20 °C.

Volume of Elution buffer to be added	For 100 Preparation	For 250 Preparation
Carrier RNA	0.8ml	2ml

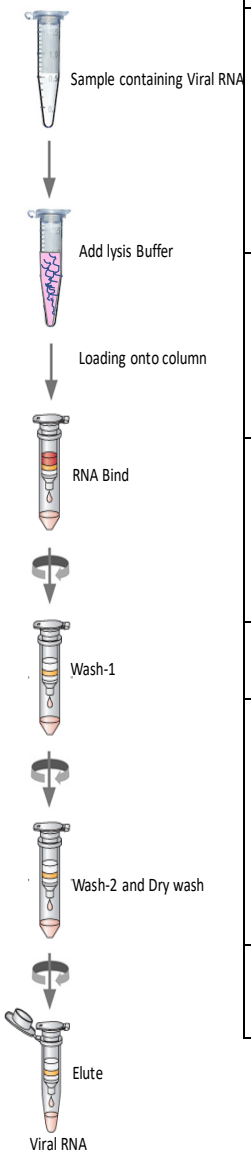
All other buffers are supplied as ready to use solutions.

RNA ISOLATION PROTOCOL

This protocol is designed for isolation of RNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

1. Prepare pre-mixture of lysis buffer and carrier RNA by adding 8 µl carrier RNA per 100 µl lysis buffer. Alternatively prepare the volume required based on the number of extractions required.
   Ex: For 5 RNA isolation, add 40 µl carrier RNA into 500 µl lysis buffer.
2. Add 100 µl of lysis buffer into microcentrifuge tube.
3. Add 200 µl VTM/sample to the lysis buffer in the microcentrifuge tube. Mix by vortexing for 15-30 sec..
   Note: To ensure efficient lysis, it is essential that the sample is mixed thoroughly with lysis buffer to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.
4. Incubate at room temperature (15–25°C) for 10 min.
   Note: Viral particle lysis is complete after lysis for 10 min at room temperature. No need for longer incubation times thus does not improve the yield or quality of the purified RNA.
5. Add 270 µl ethanol (96–100%) to the sample, and mix by vortexing for 15 sec. After mixing, briefly centrifuge the tube to remove drops from inside the lid.
6. Carefully apply entire lysed sample solution from step 5 to the spin column (in a 2 ml collection tube). Close the cap, and centrifuge at 8000 rpm for 1 min and discard the flow through.
7. Add 500 µl Wash Buffer-1and centrifuge at 8000 rpm for 1 min. Discard the flow through.
8. Add 500 µl Wash Buffer-2 and centrifuge at 12000 rpm for 1 min. Discard the flow through.
9. Remove the spin column from the tube and place it in a centrifuge tube and centrifuge at 12000 rpm for 1 min as a dry wash to remove the residual ethanol from the spin column.
10. Place the spin column in a clean 1.5 ml microcentrifuge tube and add 50 µl Elution Buffer equilibrated to room temperature. Incubate at room temperature for 1 min.
    Note: Use pre-warmed (at 56°) elution buffer.
11. The eluted RNA should be store at -80°C or below or use it immediately.

SCHEMATIC DIAGRAM OF PROCESS FLOW



TROUBLESHOOTING

S.no	Problem	Possible cause	Solution
1	Clogged Mini prep Spin column (capped)	Too much of starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use correct amount of starting material (see protocols)
2	Low RNA yield	Too much of starting material	Reduce the amount of starting material. Use the correct amount of starting material (check protocol)
3	Low Viral Load	Take the sample volume is larger than 140µl, increase the amount of lysis buffer-carrier RNA proportionally. (e.g: 280µl sample will require 1120µl lysis buffer-Carrier RNA) and use a large tube.	
4	Ethanol carryover	Before elution pre-warm the column at 55°C for 1 min	
		After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow through otherwise carryover of ethanol will occur, to eliminate any chance of possible ethanol, centrifuge the empty column for another step for 1 min at 14000rpm.	
5	DNA contamination	No DNase treatment- follow the optional on column DNase digestion protocol.	

QUALITY CONTROL

In accordance with GB's ISO-certified Total Quality Management System, each lot of the Gb's Pure Spin viral RNA Isolation Kit is tested against predetermined specifications to ensure consistent product quality.

SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS).

IVD CE

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