GB's AURA PURE
Corona Virus (Covid-19)RNA ISOLATION KIT
Magnetic Beads Based

KIT NAME	KIT SIZE	CAT. NO
GB's Aura Pure -Covid-19 viral RNA Isolation Kit	100T	RBCOV01100T
GB's Aura Pure -Covid-19 viral RNA Isolation Kit	500T	RBCOV01500T

GENUINE BIOSYSTEM

PRINCIPLE

The GB'S AURA PURE Corona virus (COVID-19) RNA Isolation kit is designed for the isolation of RNA from nasal/throat swabs collected in viral transfer medium (VTM. This kit provides reagents and magnetic beads for isolation of viral RNA from the samples. The procedure is based on the adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation with a Lysis Buffer containing chaotropic ions digestion. After precipitating with Ethanol, GB's Aura Pure Beads are added to the lysate. After magnetic separation, the paramagnetic beads are washed to remove contaminants and salts using Wash Buffers. Residual ethanol from previous wash steps is removed by air drying. Finally, highly pure RNA is eluted with low-salt elution buffer or nuclease free water.

KIT SPECIFICATIONS

GB's Aura Pure Corona virus (COVID-19) RNA Isolation Kit is designed for rapid manual and automated small-scale preparation of RNA from nasal/throat swabs collected in VTM. The kit is designed for use with GB's Aura Pure magnetic separator which can hold 12 microcentrifuge tubes for the easy isolation of 12 RNA samples in less than 30 minutes. And this kit can be easily adoptable in any 96-well magnetic separator plate or other automated magnetic separation systems. The purified RNA can be used directly as template for RT-PCR, PCR, or any kind of enzymatic reactions.

Kit Contents	100 Preparation	500 Preparation
GB's Aura Pure Beads	4ml	20ml
Lysis Buffer	10ml	50ml
Wash Buffer-1 (Concentrated)	21.5ml	108ml
Wash Buffer-2 (Concentrated)	15ml	75ml
Elution Buffer	10ml	50ml
Carrier RNA	1 vial	1 vial
Proteinase K	1 vial	1 vial

- Magnetic Stand separator will be provided at additional cost

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
- Heating block or Water Bath or thermomixer
- Vortex Mixer
- Optional- Magnetic Separator

STORAGE

GB's Aura Pure Corona virus (COVID-19) RNA Isolation Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. All the kit contents can be stored at room temperature (15-25°C) and after reconstitute, aliquot and **store Carrier RNA at -20°C. Also the Proteinase K has to be stored at -20°C upon receipt of goods.**

1.

INTENDED TO USE

GB's Aura Pure Corona virus (COVID-19) 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 AURA'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

2.

IMPORTANT INSTRUCTION

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

PREPARATION OF SAMPLE MATERIALS

This protocol is for the purification of RNA from the COVID-19 virus collected from nasal/ pharyngeal swabs collected in 3 mL of Viral Transfer Media (VTM).

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 500 Preparation
Wash Buffer 1	21.5ml	108ml
Ethanol	28.5ml	142ml
Final Volume	50ml	250ml

- **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. 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 500 Preparation
Wash Buffer 2	15ml	75ml
Ethanol	35ml	175ml
Final Volume	50ml	250ml

- **Carrier RNA**: 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 500 Preparation
Carrier RNA	0.8ml	4ml

* 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 premixture of lysis buffer working solution by adding the following components per reaction.

Components	Volume one Reaction	Volume for 10 reactions
Lysis Buffer	100 µl	1000 µl
Carrier RNA	8 µl	80 µl
Proteinase K	10 µl	100 µl
Total Volume	**118 µl**	**1180µl**

2. Add 200 µl of VTM containing nasal/throat swab sample to a 2 ml microcentrifuge tube and add 118 µl Lysis Buffer with carrier RNA and Proteinase K. Mix well by vortexing for 30 seconds and keep on shaker/ rocker for 10 minutes at at 56°C on thermomixer.

3.Add 270 µl of Ethanol and 40 µl of GB'S AURA PURE beads (Resuspend GB'S AURA PURE beads completely by inverting or rotating the bottle). Mix well by pipetting the lysate up and down for 5-6 times thoroughly and keep lysate on thermomixer for 5 minutes at 37° or Room temperature at 900 RPM.

4.Separate the magnetic beads by placing the tubes in a magnetic stand. Wait for 1 minutes till all beads are collected. While the tubes are still on the magnetic stand, remove entire supernatant by gentle pipetting without disturbing the magnetic beads.

5.Remove the tubes from the magnetic separator and Add 500µl wash buffer-1, resuspend the beads completely by vortexing and keep on thermomixer for 3 minutes at 37° at 900 RPM.

6.Separate the magnetic beads by placing the tubes in a magnetic stand. Wait for 1 min till all beads are collected. While the tubes are still on the magnetic stand, remove entire supernatant by gentle pipetting without disturbing the magnetic beads.

7.Remove the tubes from the magnetic separator and Add 500µl wash buffer-2, resuspend the beads completely by vortexing and keep on thermomixer for 3 min at 37° at 900 RPM.

8.After 2nd wash, completely remove supernatant and fully dry the beads at 65°C for 5-6 Minutes.

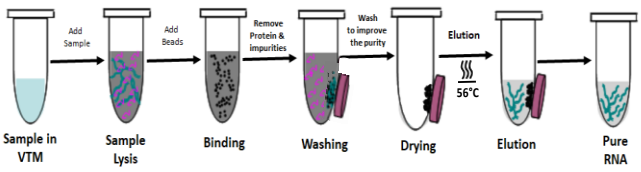
9.Add 60 µl of Elution Buffer. Resuspend magnetic beads by vertexing and incubate for 5 minutes at 56°C on thermomixer at 600 RPM speed.

10.Perform magnetic separation by keeping the tubes at magnetic separator for 1 minute.

11.Transfer the supernatant containing viral RNA to a new, nuclease free microcentrifuge tub and keep the samples in the ice or 4°C till perform the assay.

12.Use the purified viral nucleic acid in downstream application immediately or store the purified nucleic acid samples at -80°C for long term usage.

SCHEMATIC DIAGRAM OF PROCESS FLOW



Important Note:

- This kit has been specially developed for rapid Corona virus (COVID-19) RNA isolation purpose. It is recommended to use 5 µl final elute directly in to one step RT-qPCR reactions.
- This kit ideally purifies total nucleic acid (DNA and RNA) from any biological matrix. If any users are interested in removing the DNA content from the sample, they can treat the final elute with DNasae1 for 5-10 minutes and inactivate the enzyme at 70°C and use it for the RT-qPCR reactions.

TROUBLESHOOTING:

Problem	Possible cause	Suggestions
Poor yield / low sensitivity	*Incomplete sample lysis*	Sample mixed with Lysis Buffer and was not thoroughly homogenized. The mixture has to be shaken continuously. Alternatively, prolong incubation time with lysis buffer.
	Insufficient elution buffer volume	Bead pellet must be covered completely with elution buffer and needs to be fully resuspended.
	Insufficient performance of elution buffer during elution step	Remove all buffer completely from the bead pellet after the binding and wash steps. Remaining buffer decreases the efficiency of the subsequent steps.
	Aspiration of attracted bead pellet	Do not disturb the attracted beads while aspirating the supernatant. This requires special caution when removing the lysate from the beads as the lysate is usually too opaque to allow visual control of the pellet.
	Aspiration and loss of beads	Time for magnetic separation too short or aspiration speed too high.
Low purity / low sensitivity	*Insufficient washing procedure*	Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down. Use only the appropriate combinations of separator and plate, for example, GB'S AURA PURE Magnetic separator.
Poor performance of RNA in downstream applications	*Carry-over of ethanol from wash buffers*	Be sure to remove all of the 80 % ethanolic wash solution from the final wash, as residual ethanol interferes with downstream applications.
	Ethanol evaporation from wash buffers	Close buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffers from buffer reservoirs.
Carry-over of beads	*Time for magnetic separation too short*	Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.
	Aspiration speed too high (Elution step)	High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

QUALITY CONTROL

In accordance with GB's ISO-certified Total Quality Management System, each lot of the GB's Aura Pure Corona virus (COVID-19) 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

Genuine Biosystem Private Limited
Plot 97 & 98, Kattabomman street, Parvathy nagar Extn,
Old Perungalatur, Chennai-600063.TN.India
Ph:044-48681845
Email:info@gb-group.co.in
Web: www.genuinebiosystem.com