Viral RNA Isolation Kit

ARAMESH BIO GENE

Research, Development & production of

Advanced Medical Diagnosis

2022

Viral RNA Isolation Kit Hand Book

Version: 1.0 **Date of first issue: 10.27.2022** **Date of last issue:**

Designed for the isolation of Viral RNA from serum, plasma, body fluid.

Kit contents

Solution	Description	Storage	50 preps	100 preps
AVRL	Lysis Buffer	+16 to 25°C	5 ml	10 ml
AW1 (Ready to use)	Inhibitor Removal Buffer	+16 to 25°C	25 ml	50 ml
AW2 (Ready to use)	Washing Buffer 2	+16 to 25°C	30 ml	60 ml
ABB	Binding Buffer	+16 to 25°C	7.5 ml	15 ml
G solution	RNA Binding	-15 to -25°C	500 µl	1 ml
AREB	Elution Buffer	+16 to 25°C	5 ml	10 ml
Spin column	High pure	-	50 pcs	100 pcs
Collection tube	-	-	150 preps	300 preps

Storage Condition and Stability

1- All solutions of ABG Viral RNA Isolation Kit are clear and should be stored at Room Temperature (RT: +16 to +25°C).

! The buffers can show a slight yellow color. This will have no impact on the function of the buffer.

2- When precipitates have formed in solutions, warm the solutions in 56°C water bath until the precipitate dissolves.

3- Store G solution at −15 to −25°C. Repeated freezing and thawing Should be avoided.

4- All kit components are stable until the expiration date on the kit box, without showing any reduction in performance.

5- Improper storage at +2 to 8°C or -15 to -25°C will adversely impact nucleic acid purification because solutions might be precipitated.

Additional Equipment (not provided)

1- 1.5- or 2.0-mL micro-centrifuge tubes

2- Pipettes and filter tips (RNase free)

3- Standard tabletop microcentrifuge capable of 17,000 xg centrifugal force

4- Vortex mixer

5- Personal protection equipment (lab coat, gloves, goggles)

Product description

ABG Viral RNA isolation kit is designed for rapidly and easily isolation of viral RNA from a variety of sample sources including serum, plasma and body fluids.

This kit employs a proprietary lysis buffer in combination with spin column membrane to efficiently purify viral RNA from the biological sample. The protocol provides a simple method to achieve the rapid isolation of highly purified viral RNA from up to 250 µl specimen and takes only 20 minutes for complete preparation. The procedure can be used for isolation of viral RNA from a broad range of RNA viruses. However, performance cannot be guaranteed for every virus species and must be validated by the costumer.

The amount of purified viral nucleic acid depends on the sample type, the virus titer, sample source, transport and storage.

Sample Materials:

Serum

Plasma

Nasopharyngeal swab

Oropharyngeal swab

Body fluids

Quality Control

All components of ABG Viral RNA Isolation Kit are manufactured in strictly clean conditions, and their degree of cleanliness is monitored periodically. To maintain consistency, a quality control process has been carried out thoroughly from lot to lot and only the approved qualified kit will be delivered.

Yields of viral RNA isolated from biological samples are normally less than 1 µg and therefore difficult to determine photometrically. Quantitative RT-PCR is recommended for determination of Viral RNA yield.

Warning and precautions

1- Wear disposable gloves, laboratory coats and eye protection when handling specimens as if potentially infectious and reagents.
2- Wash hand thoroughly after handling samples and reagents.
3- Use sterile, disposable plastic wares and filtered pipette tips.
4- Buffers of the kit contain irritants which are harmful when contact with skin and eyes, or when inhaled and swallowed. Avoid o contacting the lysis buffer and wash buffers with acidic solution and bleach.
5- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Areas. Do not return samples, equipment and reagents in the area where you performed in previous steps.
6- Safety Data Sheets (SDS) are available online.

Before you begin

1. All centrifugation steps are carried out at room temperature (15 to 25°C).
2. Sample should be equilibrated to room temperature.
3. Check all reagent for any precipitation. If Lysis Buffer forms precipitate, please warm the it in a 56°C water bath until the precipitate's dissolves.
4. Use fresh material to avoid degradation of Viral RNA

Storage of samples

Sample type	Short term storage	Long-term storage
Serum	48 hours (+2 to +8°C)	-70°C
Plasma	48 hours (+2 to +8°C)	-70°C
Body fluids	48 hours (+2 to +8°C)	-70°C

❖ Protocol for Viral RNA Isolation

RNA Lysing Step

1- To a nuclease free 1.5 ml microcentrifuge tube:

! Working solution: G solution and AVRL can be mix thoroughly before use. In case of using working solution, add 260 µl working solution and 250 µl sample.

a. Add 250 µl sample

b. Add 250 µl AVRL

c. Add 10 µl G solution

2- Mix immediately by pulse-vortex (20 seconds) and incubate at RT (+16 to +25°C) for 10 minutes.

Binding Step

3- Add 175 µl of ABB and mix well by pulse-vortex (20seconds).

! In the presence of undigested or precipitated remnants centrifuge at 10,000 rpm is recommended. Use supernatant for the next step.

4- Assemble one spin column in to one collection tube.

5- Pipette the liquid sample in to the upper reservoir of the spin column.

6- Centrifuge for 30 seconds at 12,000 rpm.

7- Remove the spin column from the collection tube and discard the flow through liquid, and the collection tube.

8- Assemble the spin column with a new collection tube.

Washing Steps

9- Add 500 µl AW1 to the upper reservoir of the spin column.

10- Centrifuge for 30 seconds at 12,000 rpm.

11- Remove the spin column from the collection tube and discard the flow through liquid, and the collection tube.

12- Assemble the spin column with a new collection tube.

13- Add 600 µl AW2 to the upper reservoir of the spin column.

14- Centrifuge for 1 minute at 12,000 rpm and discard the flow through.

15- Centrifuge for 3 minutes at 14,000 rpm to remove residual ethanol.

16- Remove the spin column from the collection tube and discard the collection tube.

Elution Step

17- Insert the spin column into a clean, sterile 1.5 ml microcentrifuge tube.

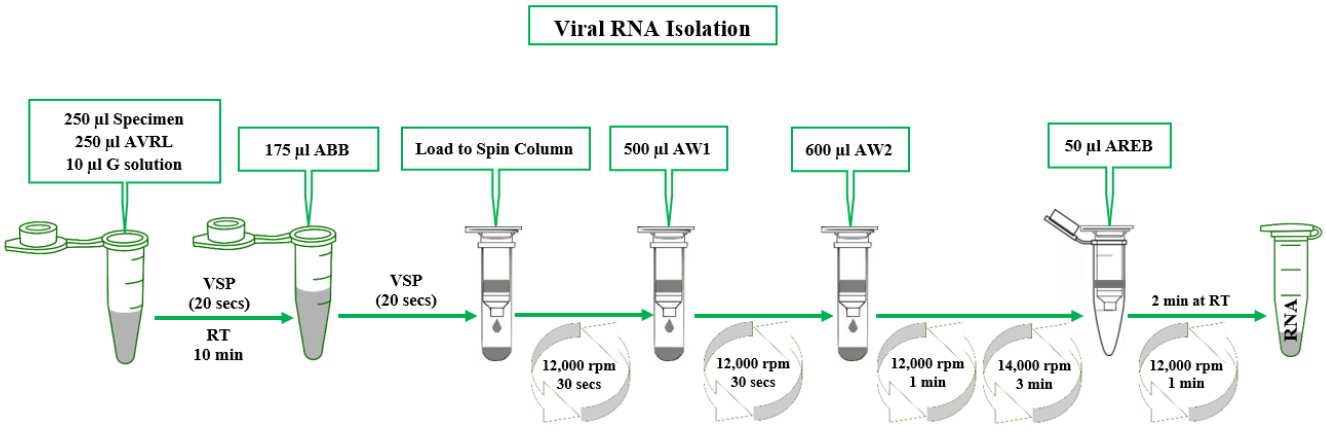
18- Add 50 µl of AREB to the upper reservoir of the spin column and incubate at RT (+16 to +25°C) for 2 minutes.

19- Centrifuge for 1 minute at 12,000 rpm.

20- Use the eluted Viral RNA directly or store it -20°C for short term use.

21- For later analysis store eluted RNA at -70°C.

Viral RNA Isolation chart:



Troubleshooting

This troubleshooting guide may be helpful for solving any problems that may arise. However, if you have questions or experience problems with this product. Please contact our Technical Support staff. Our scientists are committed to provide rapid and effective assistance.

Observation	Cause	Comment
low nucleic acid concentration	Kit stored under suboptimal conditions	Store kit contents according to the labeled temperature
	Buffers or other reagents were exposed to conditions that reduced their effectiveness	Store all buffers at +16 to +25 ℃
		Close all reagents bottles tightly, to preserve pH, stability, …
		Aliquot and store G solution at -20°C
	Incomplete cell lysis because of insufficient mixing with AVRL	Repeat the extraction procedure with a new sample. Mix the sample and AVRL immediately and thoroughly by pulse-vortex (Recommended 45 seconds).
	Incomplete lysis because of insufficient incubation time	Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.
	Clogged spin Filter (Inefficient disruption or homogenization)	• Increase lysis time • Increase centrifugation Reducing amount of starting material
	Reagents and samples not completely mixed	Mix the sample tube completely after addition of each reagent.
	High level of RNase activity	Be careful to create an RNase-free working environment.

		Process starting material immediately or store at -80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store immediately at -80°C.
	Suboptimal reagent has been used for elution. Alkaline pH is required for optimal elution	Do not use water to elute nucleic acids from spin column.
		Use the elution buffer from the kit.
Incompletely or no restriction enzyme cleavage product.	Glass fibers which can co-elute with nucleic acid, scatter light	After elution step is finished, remove the spin column, and centrifuge the tube containing eluted sample for 1 minute at maximum speed. Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of original tube
Absorbency (A_{260}) reading of product is too high.	Glass fibers which can co-elute with nucleic acid, scatter light	See suggestion under “Incompletely or no restriction enzyme cleavage product.” above.

Contact and Support

Aramesh Bio Gene appreciates its customers, and strives to make their experience the best it can be. Ask technical questions about all AB Gene products, from product choice, to product use. AB Gene support team composed of highly trained experienced scientists, who are able to troubleshoot most problems you may encounter.

Contact our technical support at any time by selecting one of these ways:

- phone: +9821- 22142231/22142883
- Email: info@abiogene.ir
- Company address: 1st floor, No. 11, Majd street, East Sarv street, Kaj Square, Sa'adatabad, Tehran, Iran.