ROJETECHNOLOGIES

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RNJia Virus Kit

Viral RNA isolation based on silica technology

- MiniPrep

For Viral RNA Isolation from

Body Fluid

Serum

Plasma

By ROJE

Edition, 01/2022

ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable Molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

Contents

Kit Content

Component	100 preps
BFC	20ml
BWB1 (concentrate)	22ml
BWB2 (concentrate)	16.5ml
ERR	10ml
Carrier RNA	620µg
HiPure DR Column	100
Collection Tube	200

Storage

Shipment condition is checked by ROJETechnologies. After arrival, all reagents should be kept dry, at room temperature. , Carrier RNA is storable at room temperature before preparation. However, after adding ERR buffer, it is recommended that it must store at -20°C and be aliquoted to 100µl volumes and avoid frequent freeze-thaw. When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

RNJia Virus Kit provides the components and procedures necessary for purifying viral RNA from cell-free samples such as body fluid, serum, plasma etc. RNJia Virus Kit is intended for molecular biology applications. This kit has IVD certificate to be used for SARS-COV-2 isolation. We recommend all users to study RNA experiments guideline, before starting their work.

Guarantee & Warranty

ROJETechnologies guarantees the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any product does not satisfy you, due to reasons other than misuse, please contact our technical support team. If problem is due to manufacturing process, ROJE team will replace the Kit for you.

Notice to Purchaser

This product is only for experiments and not for commercial use in any kind. No right to resell this kit or any components. For information about our licensing or distributors contact ROJE business team.

Warning and Precautions

Due to chemical material usage that may be hazardous, always make sure to wear suitable lab coat, disposable gloves, and protective eyewear. Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.rojetechnologies.com.

Quality Control

RNJia Virus Kit is tested against predetermined experiments on a lot-to-lot basis according to ROJETechnologies ISO-certified quality management system, to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on web at www.rojetechnoloes.com.

Description

The RNJia Virus Kit is designed for rapid and efficient purification of high-quality viral RNA from various human and animal liquid samples such as body fluid, plasma, serum etc. The kit utilizes a silica-based membrane technology in the form of a convenient spin column, which takes less than 25 minutes. RNJia Virus Kit needs less handling and it is convenient for simultaneous isolation, which makes it favorite for laboratories with many isolations in a day. The purified RNA is free of proteins, nucleases, and other contaminants or inhibitors of downstream applications. Isolated RNA can be directly used in PCR, qPCR or other nucleic acid-based assays.

Procedure

RNJia Virus Kit is designed for isolating RNA from body fluid, serum, plasma etc. Lysis is achieved by incubation of the sample in BFC buffer. Appropriate conditions for RNA binding to the silica membrane is achieved by the addition of ethanol to the lysate. Then, RNA is selectively bound to the membrane. Contaminants are removed by two specific washing buffers. Pure viral RNA is finally eluted in rehydration buffer. Isolated RNA is ready to use in downstream applications.

Equipment & Reagents to Be Supplied by User

- Ethanol (%96-100)
- Sterile, RNase-free pipets and pipet tips
- 1.5ml Microtube
- Vortex

- Centrifuge and Micro centrifuge
- Dry Heat Block/ Water Bath

Applications

The isolated RNA can be used in many downstream applications:

- Different kinds of PCRs
- Restriction enzyme digestion
- Viral genotyping
- Viral detection
- Viral load monitoring

Features

Specific features of RNJia Virus Kit are listed here in Table 1.

Table 1. RNJia Virus Kit features and specifications

Features	Specifications
Elution volume	60µl
Technology	Silica technology
Main sample type	Body Fluid, serum, Plasma
Processing	Manual
Sample amount	Up to 200µl
Biomolecule isolation	RNA
Operation time per reaction	Less than 25 Min
Typical yield	Varies
Carrier RNA	provided in the kit

Recommended Starting Material

The size of recommended starting material to use with determined lysis volume are listed here.

Table 2: Recommended starting material and Lysis Buffer amount

Sample	Size of Starting material	Lysis Buffer Amount
Plasma/serum	200µl	200µl

Protocol

Isolation of Viral RNA (based on silica technology)

Sample type:

- Body fluid
- Serum
- Plasma

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Equilibrate samples to room temperature.
- Equilibrate ERR to room temperature for elution step.
- Add carrier RNA reconstituted to BFC according to following instructions.
- Before starting, add ethanol (%96–100) to a bottle containing BWB1[1] and BWB2 concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been added. Store reconstituted BWB1 and BWB2 at room temperature (15–25°C). Refer to washing buffer preparation section.

  Note: Always mix reconstituted Buffer BWB1 by shaking before starting the procedure.

- Add ethanol (%96–100) to a bottle containing Buffer BWB2 concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been added. Store reconstituted BWB2 at room temperature (15–25°C). Refer to washing buffer preparation section.

  Note: Always mix reconstituted BWB2 by shaking before starting the procedure.

  If BFC forms precipitate, please warm it up to 56°C until the precipitate has fully dissolved.

Washing Buffer Preparation

Before the first use, add appropriate amount of ethanol (%96-100) to the washing buffer, then mix thoroughly to have prepared washing buffer, refer to the bellow Table. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

Table 3: Washing buffer preparation

[1] Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach

Buffer Name	Reaction rates	Concentrated Volume	Amount of Ethanol	Final Volume
BWB1	100 Preps	22ml	33ml	55ml
BWB2	100 Preps	16.5ml	38.5ml	55ml

Addition of carrier RNA to BFC[2]

RNA Carrier preparation

Add 620µl ERR to the tube containing 620µg lyophilized carrier RNA to obtain a solution of 1µg/µl Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Important! Note that carrier RNA does not dissolve in BFC. It must first be dissolved in ERR and then added to BFC.

BFC preparation

Calculate the volume of Buffer BFC–carrier RNA mix needed per batch of samples to be simultaneously processed from Table 4.

[2] Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach.

Table 4. Volumes of BFC and carrier RNA–ERR mix required for the procedure.

Samples Number	BFC amount (ml)	Carrier RNA–ERR amount(µl)	Samples Number	BFC amount (ml)	Carrier RNA–ERR amount (µl)
1	0.2	5.6	13	2.6	72.8
2	0.4	11.2	14	2.8	78.4
3	0.6	16.8	15	3	84.0
4	0.8	22.4	16	3.2	89.6
5	1	28.0	17	3.4	95.2
6	1.2	33.6	18	3.6	100.8
7	1.4	39.2	19	3.8	106.4
8	1.6	44.8	20	4	112.0
9	1.8	50.4	21	4.2	117.6
10	2	56.0	22	4.6	123.2
11	2.2	61.6	23	4.8	128.8
12	2.4	67.2	24	5	134.4

Attention! Mix gently by inverting the tube 10 times. To avoid foaming, do not vortex.

Note: The sample-preparation procedure is optimized for 5.6µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing BFC.

Process

- Pipet 200µl prepared BFC containing carrier RNA into a 1.5ml clean microcentrifuge tube. Add 200µl plasma, serum, cell-culture supernatant or cell-free body fluid to the Buffer BFC–carrier RNA in the microcentrifuge tube. Mix by pulse vortexing for 15 s.

  Note: If the sample volume is larger than 200µl, increase the amount of Buffer BFC–carrier RNA proportionally (for example, a 300µl sample will require 300µl Buffer BFC–carrier RNA, BFC can be ordered separately) and use a larger tube.

  Note: It is important that the sample is mixed thoroughly with BFC to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.

- Incubate at room temperature (15–25°C) for 10 min.

  Attention! Viral particle lysis is completed after lysis for 10 min at room temperature. Longer incubation times have no effect on the yield or quality of the purified RNA.

- Centrifuge briefly the tube to remove drops from the inside of the lid. Add 200µl ethanol (%96–100) to the sample, and mix by pulse vertexing for 15s. Then, centrifuge the tube to remove drops from inside the lid. To ensure efficient binding, it is essential that the sample is mixed thoroughly with the ethanol to yield a homogeneous solution.

  Attention! Use only ethanol, since other alcohols may result in reduced RNA yield and purity. Do not use denatured alcohol, which contains other substances such as methanol.

  Note: If the sample volume is greater than 300µl, increase the amount of ethanol proportionally (for example a 280µl sample will require 300µl ethanol).

- Gently, transfer all of the mixture to a HI Pure DR column placed in a collection tube, without wetting the rim (supplied in the kit box). Centrifuge at 6000 x g (8000 rpm) for 1 min. Discard flow-through and place back the HI Pure DR column in to the collection tube.
  Note: Close each spin column to avoid cross-contamination during centrifugation.

  Note: If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all of the solution has passed through.

- Repeat previous step until all of the lysate has been loaded onto the spin column.
- Add 500µl BWB1 to HiPure DR column. Centrifuge at 6000 x g (8000 rpm) for 1 min. Discard flow-through and place back the HiPure DR column in to the collection tube.

  Note: It is not necessary to increase the volume of BWB1 even if the original sample volume was larger than 200µl.

- Add 500µl BWB2 to HiPure DR column, then centrifuge at full speed 20,000 x g (14,000 rpm) for 3 min.

  Note: Residual BWB2 in the eluate may cause problems in downstream applications. Place the HiPure DR column in a new collection tube (not provided), centrifuge at full speed for 3 min and discard the old collection tube with the flow-through. Centrifuge at full speed for 1 min.

- Place the HiPure DR column in a clean 1.5ml microcentrifuge tube (not provided). Discard the old collection tube containing the flow-through. Add 60µl ERR equilibrated to room temperature into the HiPure DR column close the cap, and incubate at room temperature for 1 min.

- Centrifuge at 6000 x g (8000 rpm) for 1 min.

  Note: A single elution with 60µl ERR is sufficient to elute at least 90% of the viral RNA from the HiPure DR column. Performing a double elution using 2 x 40µl ERR will increase

yield by up to 10%. Elution with volumes of less than 30µl will lead to reduced yields and will not increase the final concentration of RNA in the eluate.

Note: Viral RNA is stable for up to one year when stored at –30 to –15°C or at –90 to –65°C.

symbols

symbols	meaning	symbols	meaning
	Hazardous chemicals		manufacturer
	Date of manufacture		Temperature limitation
	Use by	LOT	Lot number
REF	Reference number	IVD	In Vitro Diagnostics

Troubleshooting

Here we try to cover as many problems as you may see in using this product, however scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

	Symptoms	Problem	Suggestion
Viral RNA Isolation Problems	a. Low RNA Yield	• Carrier RNA is degraded	• After reconstitution in ERR, not stored at -20℃ or freeze-thaw frequently • BFC-Carried buffer is stored at 28℃ more than 48h. In each case, reconstitute RNA carrier in BFC-Carried buffer again and prepared new BFC. Then, repeat the procedure.
		• Carrier RNA not added to BFC	• Reconstitute carrier RNA in ERR and mix with BFC as described before. Repeat the purification process with new samples.
		• Low virus concentration	• Concentrate the sample volume to 140µl using a micro concentrator. Repeat the isolation with new sample.
		• Samples are not fresh	• Always use fresh samples • Do not freeze and thaw sample more than once
		• Inefficient protein denaturation	• Precipitate, formed in Buffer BFC–carrier RNA after storage at 2–8°C. Redissolve the precipitate and repeat the isolation with new sample
		• Precipitate in BFC	• Heat the BFC buffer up to 80°C and then use it.
		• RNase contamination in BFC buffer	• Discard the contaminated buffer. Repeat the isolation with new sample and new stock of BFC–carrier RNA buffer
		• RNA degraded	• Ensure that the samples are processed quickly. If necessary, add RNase inhibitor to the sample

			to stop RNA degradation in starting material.
		• Forget to add ethanol to lysate	• Repeat the procedure with new sample.
		• Low percentage of ethanol	• Make sure you are using 96–100% Molecular biology grade ethanol.
		• BWB1 and BWB2 prepared incorrectly	• Referred to some tips to know, check the BWB1 and BWB2 dilution process and repeat the procedure again.
		• BWB1 and BWB2 used in wrong order	• Make sure to use BWB1 and BWB2 in right order (according to the protocol).
	b. DNA contamination	Samples containing cells	• Samples containing cells should be made cell-free by centrifugation or filtration. • If using centrifugation, pellet the cells for 10 min at 1500 x g and use supernatant for isolation of viral RNA.
General Problems	c. RNA does not perform well in downstream applications	Too much carrier RNA in the eluate	• Determine the maximum amount of carrier RNA suitable for your RT-PCR. Adjust the concentration of carrier RNA added to BFC
	d. Column clogging	Precipitates were not removed.	• Do not use membrane frozen and thawed more than once
		Lysate not completely passed through the membrane	• Centrifuge for 1 min at full speed or until all the lysate has passed through the membrane.

Ordering Information

Category	Product name	Cat NO.	Size
RNA Technologies	**RNJia Virus Kit**	RN983072	100 preps
Related products	**DNall VirAll Kit**	DN983053	100 preps
	DNJia Virus DNA Kit	DN983056	100 preps
	DNJia VirAll Kit	DN983053	100 preps

Technical Assistance

ROJETechnologies guarantees your complete satisfaction. ROJE technical support team composed of highly experienced scientists, who are able to troubleshoot most problems you may face. Our technical support team can offer expert advice which help you select suitable product.

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

- Through our telephone and fax number; +982191070705.
- You can submit your question directly to ROJE technical support team from our website (www.rojetechnologies.com).
- Or send your questions to this email address, technicalsupport@rojetechnologies.com.

Appendix 1: Handling RNA

RNA is highly sensitive to degradation due to the ubiquitous presence of RNases in the environment. unlike DNases, which require metal ions for activity, RNases do not require cofactors for their enzymatic activity and can maintain activity even after prolonged boiling or autoclaving. Since RNases are active enzymes, and are too hard to be deactivated, do not use any plasticware or glassware without first removing possible RNase pollution. Special precautions should be taken when working with RNA. All reagents and equipment must be specially treated to inactivate RNases prior to use.

The following are some tips and techniques to remember when working with RNA:

General Tips

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory

mobilizations. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.

- Treat surfaces of benches with commercially available RNase inactivating agents such as RNZO (Order by cat No RN983018 and RN983019).
- Treat non-disposable glassware and plasticware before use, to ensure that it is RNase-free. Thoroughly rinse plasticware with 0.1N NaOH/1mM EDTA, and RNase-free water.
- Use disposable RNase-free plasticware to decrease the possibility of sample contamination.
- Keep purified RNA on ice when aliquots are pipetted for downstream applications.
- Glassware should be immersed in freshly prepared %0.1 (v/v) DEPC in water or Ethanol for several hours, followed by draining and autoclaving.

Appendix 2: RNA Storage Condition

RNA samples are commonly stored at −20 °C or −80 °C, eluted in RNase-free water. Under these conditions, RNA is not degraded after 1 year.

RNA Quality

The ratio of absorbance at 260nm and 280nm is used to assess the purity of RNA using UV absorption, by a spectrophotometer or Nanodrop. For pure RNA, A260/A280 ratios should be around 2.1. If the ratio is noticeably lower in either case, it may indicate the presence of protein or other contaminants that absorb strongly at or near 280nm. High A260/A280 purity ratios are not indicative of an issue.

Appendix 3: RNA Integrity

The integrity of RNA molecules is important for experiments that try to reflect the snapshot of gene expression of RNA isolation. The least expensive method for checking RNA integrity is to run the RNA on a %1 DGE agarose gel and examine the ribosomal RNA (rRNA) bands. The upper ribosomal band (28S in eukaryotic cells and 23S in bacterial cells) should be about twice the intensity of the lower band (18S in eukaryotic cells and 16S in bacterial cells) and should be sharp and tight.

Denaturing agarose gel electrophoresis

Formaldehyde is used to keep the RNA from denaturation, and when RNA is heated in the presence of formaldehyde all secondary structures are eliminated. Therefore, gel electrophoresis of RNA in agarose gels containing formaldehyde provides a good denaturing

gel system. Formaldehyde is a potential carcinogen. Take appropriate safety measures and wear gloves when handling.

Gel preparation

- Prepare % 1.2-2 gel with TAE 1x buffer (%5 formaldehyde).
- Pour the gel using a comb that will form wells large enough to accommodate at least 20µl.
- Assemble the gel in the tank.
- Use TAE 1x buffer (%5 formaldehyde) as the tank buffer.

RNA Sample preparation

- Add 10µg of purified RNA to 6µl of ROJE sample buffer (in RNA Loading Set, order by cat No LD983007) and incubate at 70°C for 3min.
- Add 3µl Hinari to the mixture of RNA and sample buffer and mix well by pipetting.
- Then run the mixture in the prepared gel.

Electrophoresis

Load the gel and electrophorese at 5-6 V/cm until the loading dye at least has migrated 2/3 the length of gel.

Results

Visualize the gel on a UV transilluminator. The 28S and 18S ribosomal bands should be sharp and intense. The intensity of the 28S band should be twice more than the 18S band. The following Figure shows different RNA samples, isolated by RNJia kit and run by DGE.

Note: If DNA contamination is present, the high molecular weight smear will be appeared above the 28S band.

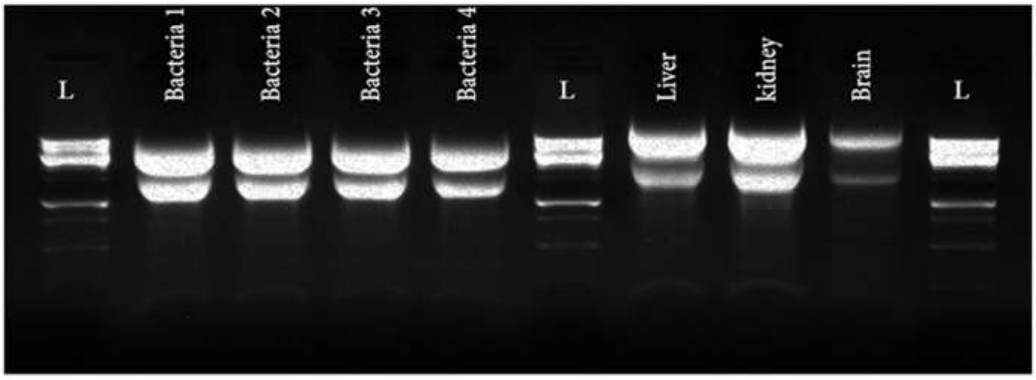

Figure1. Isolated RNA using RNJia Kit, RNA was isolated from the indicated animal tissues and bacteria cells using the RNJia Kit. For each sample, 10µg RNA was pretreated by ROJE Sample Buffer and analyzed on Denaturing Gel Electrophoresis (DGE).

Appendix 4: DNA Contamination

Different methods exist for monitoring genomic DNA contamination, for example, using OD measurement or via agarose gel electrophoresis. Nevertheless, genomic DNA traces which are not detectable by the above methods, can produce nonspecific results in sensitive downstream applications. The co-amplification of cDNA and contaminating genomic DNA produce one or more additional PCR fragments that can be traced via agarose gel electrophoresis.

strategies to solve the problem:

- Design PCR primers for span exon boundaries, which includes one or more intronic sequence motifs in the genomic DNA, results in amplification of cDNA template.
- Design a minus control reaction with reverse transcriptase. In that specific reaction, instead of the reverse transcriptase enzyme, water is added to the reaction mixture. PCR product in this control reaction indicate genomic DNA contamination.

Appendix 5: Convert RPM to RCF (centrifuge)

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.

Factory address

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ROJETECHNOLOGIES has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable Molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

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