FOREGENE
EXPERT IN NUCLEIC ACID EXTRACTION

Viral RNA Isolation Kit

Cat.No.RE-02011/02014

For purification of viral RNA from plasma,serum,cell-free body fluids,cell-culture supernatants

For research use only

Store at room temperature

Index

Product introduction

This kit adopts centrifugal columns and formulas developed by our company to efficiently isolate and purify viral RNA molecules from samples such as plasma, serum, cell-free body fluids, and cell culture supernatant. The kit is specially added with Linear Acrylamide, which can easily capture trace RNA molecules from the system, which is convenient, fast, with high yield and good reproducibility. RNA-Only Column can efficiently bind to RNA. With the help of the unique formula, it can process a large number of samples at the same time.。

The whole system is RNase free, for ensuring that RNA molecules are intact without degradation; Buffer viRW1 and Buffer viRW2 rinsing system make it possible for obtained RNA molecules free from proteins, DNA molecules, nucleases, and other impurities contamination, which can be directly used in downstream experiments

Product characteristics

- Room temperature (15-25 ° C) operation, no need for ice bath and low temperature centrifugation
- The entire kit is RNase-Free, no need to worry about RNA degradation
- High RNA yield: The partnership of RNA-Only Column and unique formula can efficiently purify RNA molecules
- Fast speed: easy operation, can be done within 30 minutes
- Safety: No organic reagent required
- High quality: The viral RNA isolation is of high purity, free of proteins and other impurities contamination, and can meet various downstream experimental applications

Kit application

Suitable for extraction and purification of viral RNA molecules in samples such as plasma, serum, cell-free body fluids and cell culture supernatants

Viral RNA application

Viral RNA purified by the Viral RNA Isolation Kit can be used in various downstream molecular experiments, such as: RT-PCR, Real Time PCR, Northern Blot, microarray analysis, molecular cloning, etc

RNA storage

It is recommended to use RNase-Free ddH2O to elute RNA molecules for immediate downstream experiments or store RNA molecules at -80 ℃. RNA molecules can be stored for one year at -80 ℃

The control of product qualification

According to FOREGENE ’s Total Quality Management System standard, each batch of viral RNA isolation kits is strictly tested multiple times to ensure the reliability and stability of each batch of kits.

Kit contents

Viral RNA Isolation Kit		
Kit composition	RE-02011	RE-02014
	50 times	200 times
Linear Acrylamide	120μl	480μl
Buffer viRL*	25ml	100ml
Buffer viRW1*	25ml	100ml
Buffer viRW2	24ml	96ml
RNase-Free ddH_2O	10ml	40ml
RNA-Only Column	50 sets	200 sets
instructions	1 serving	1 serving

*：Buffer viRL and Buffer viRW1 contain irritating chaotropic salts. Please wear gloves and take relevant protective measures during operation.

Product information

Type	Centrifugal column	Purification preparation	Foregene centrifugal column,reagent
Flux	1-24 samples	Preparation time	~30min (24 samples)
Centrifuge	Desk centrifuge	Maximum liquid capacity of centrifugal column	800μl
Load capacity of RNA Purification column	20μg	Size of single sample Handling	≤200μl
Minimum volume of elution	30-50μl	Yield	≥80% recovery efficiency

Storage requirements

This kit can be stored for 24 months under room temperature (15-25 ℃) and dry

conditions. If it needs to be stored for a longer time, it can be stored at 2-8 ℃. Linear Acrylamide solution can be stored frozen at -20 ℃; Buffer viRL can be stored at 2-8 ℃ for up to 48h after adding Linear Acrylamide.

Note: If you keep it at a low temperature, the solution is prone to precipitate. The solution in the kit must be left at room temperature for a period of time before use. If necessary, it can be preheated in a 37 ° C water bath for 10 minutes to dissolve the precipitate and mix it before use.

Kit component

- Linear Acrylamide：Reduce background adsorption of purification columns and easily capture trace RNA molecules in the system。
- Buffer viRL：Provide the environment required for sample lysis and the column.
- Buffer viRW1：Remove impurities such as proteins and DNA molecules from RNA molecules。
- Buffer viRW2：Removes the residual salt ions from RNA molecules.
- RNase-Free ddH_2O：Elute viral RNA molecules on Membranes of Purified Column.
- RNA-Only Column：Specific adsorption of RNA fragments.

RNA-Only Column characteristics

Maximum binding capacity	20μg
Maximum loading volume	800μl
RNA size distribution	RNA≥200nt
Minimum elution volume1*	30μl
Selection of samples	Plasma, serum, cell-free body fluid or cell culture supernatant,
Maximum amount of starting material 2*	200μl

1*：The minimum elution volume of 30 μl is a reasonable recommended volume, given that the balance is well-kept between RNA recovery and concentration. If you want to increase the yield of RNA molecules, you can increase the eluent volume appropriately；For the purpose of increasing the concentration of the purified RNA, the volume of the eluent should be appropriately reduced while sacrificing the yield of a portion of RNA molecules. For example, an elution volume of 15 ul is used to obtain a higher concentration of RNA molecules.

2*：For larger sample sizes, use multiple RNA-Only Columns for RNA purification.

RNA extraction rate and purity

Viral RNA molecules can be purified and obtained by using Viral RNA Isolation Kit from samples such as plasma, serum, cell-free body fluids, and supernatants of cell cultures.

The yield is related to the sample itself, the initial amount of the sample, the freshness of the sample, the sample storage time, and relevant operations. For purified RNA, the value of OD260 / 280 ranges from 1.8 to 2.1

Caution: (be sure to read the notes carefully before using)

- All experimental steps are performed at room temperature (15-25 ° C) (including centrifugation). Do not use ice bath and low temperature (4 ° C) centrifugation.
- Avoid repeated freezing and thawing of samples, otherwise the extracted viral RNA molecules will be degraded and the amount of extraction will also decrease.
- Do not handle more than 200 μl of sample, otherwise it will affect the yield and the purity of viral RNA molecules.
- After adding Linear Acrylamide to Buffer viRL, it can be stored at 2-8 ℃ for a maximum of 48 hours. Please prepare for it when you need it.
- Before using the kit, please add anhydrous ethanol to Buffer viRW2. Please refer to the label on the reagent bottle. See the table below for the volume of anhydrous ethanol in different kits：

Product specification	The amount of anhydrous ethanol
RE-02011	60ml
RE-02014	240ml

- RNA yield and quality are related to elution volume and sample processing volume. It is recommended to use 200 μl sample per 500 μl Buffer viRL.
- Elution volume: The volume of eluent should not be less than 30 μl, otherwise it will affect the efficiency of RNA recovery.
- Please check whether there is presence of crystallization in Buffer viRL and Buffer viRW1 in the kit. If there is crystallization after storage at low a temperature, you

can place the buffer at room temperature or at 37 ° C for a period of time, dissolving the crystals and mix them before use.

Preparation before operation

Please read the instructions carefully before using this kit. The viral RNA isolation kit is simple, convenient, and fast. The instructions provide the correct use of the entire kit. Please prepare for the necessary experimental materials and equipment before use.

Experimental materials and equipment

- 200 μl of fresh or frozen plasma, serum, cell-free body fluid or cell culture supernatant。
- Desk centrifuge(≥13,400×g)、Pipettes, etc.
- Sterile RNase-Free centrifuge tubes, pipette tips, etc

Bring your own reagent

- Anhydrous ethanol.
- Isopropanol

Security

- This product is for scientific research use only. Do not use it for medicine, clinical medicine, food and cosmetics purposes.
- When using this kit, please wear lab clothes, disposable latex gloves, disposable masks, etc. to protect yourself; and avoid artificially introduced RNase pollution。
- Buffer viRL contains chaotropic salts: denaturant. Be aware of its irritant.
- Buffer viRW1 contains chaotropic salts: denaturant. Be aware of its irritant.
- Buffer viRW2 contains ethanol: flammable.

Operation guide

The kit is operated at room temperature (15-25 ° C) at all times. Do not use ice bath and low-temperature centrifugation.

Sample selection and preservation

Plasma and serum can be stored at 2-8℃ for 6 hours after collection. If long-term storage is required, it can be stored at -20 ℃ or -80 ℃. Avoid repeated freezing and thawing of samples during storage (no more than once), otherwise the yield of RNA molecules will decrease. Additionally, the frozen proteins formed during the freeze-thaw processes can also block the adsorption column. Therefore, If the condensed frozen protein is visible to the naked eye after the sample is thawed, centrifuge at 6800 ×g for 3 minutes. Carefully aspirate the supernatant。

Preserve samples in Buffer viRL

RNA molecules will not be degraded by RNase in Buffer viRL. If samples from plasma, serum, cell-free body fluid and cell culture supernatant are not used immediately after lysis with Buffer viRL, they can be stored at room temperature for about 24 hours and at 4 ° C for about 1 week. For longer storage, please store at -80 ℃. Please place the solution at room temperature or at 37 ℃ for dissolving purposes.

Initial sample size

The correct initial processing size of the sample is necessary for optimal RNA yield and purity. The maximum amount of sample processing is related to the following factors:

- ❖ The type of sample itself and the abundance of sample RNA molecules；
- ❖ The amount of buffer viRL determines the effective lysis of the sample；
- ❖ The ability of RNA-Only Column to specifically bind to RNA molecules.

Based on the above factors, we recommend that the initial processing volume of the sample should not exceed 200μ. If the processing volume of the sample is too large, Buffer viRL cannot lyse the sample completely, resulting in low purity of the purified RNA. At the same time, it may exceed the maximum loading capacity of the RNA-Only Column and waste precious samples

Materials treatment instructions

Plasma, serum, cell-free body fluids or cell culture supernatants: single treatment, do not exceed 200μl。

RNase contamination preservation

- Human contact is an important source of RNase contamination, and some reagents might have an irritating odor. Please change gloves frequently during the operation and wear disposable masks
- Use RNase-free tips and other plastic products
- The RNA molecule will not be degraded by RNase when it is in Buffer viRL, but RNase-free plastic and glassware should be used during the following processing after extraction. Glassware can be baked at 150 ° C for 4 hours, plastic products can be soaked in 0.5M NaOH for 10 minutes, and then thoroughly washed with water and autoclaved to remove RNase
- Use RNase-free water to prepare the solution (Add water to a glass bottle that has been treated without RNase, then add DEPC to a final concentration of 0.01% (v / v). Mix well and leave it overnight, autoclave)

Genomic DNA contamination and removal

Viral RNA isolation kit is mainly used to obtain viral RNA molecules from samples such as plasma, serum, cell-free body fluid and cell culture supernatant. Purified RNA

molecules are usually directly used for downstream operations without the need for DNase treatment.

Due to the fact that there might be trace amounts of DNA molecules in the sample, it is very sensitive to some RNA analysis experiments, such as: Real-time RT-PCR analysis of low-abundance genes. At this time, you can use appropriate DNase to further remove DNA contamination

Operation procedure (Room temperature (15-25 ° C) operation, never ice bath and low-temperature centrifugation)

Before use, please add anhydrous ethanol to Buffer viRW2, and refer to the label on the bottle for the volume.

1. Using a pipette, add 500μl Buffer viRL and 2μl Linear Acrylamide to a clean 2ml centrifuge tube, mixing by inverting the tube upside down
   Note: To avoid foaming of the solution, do not use vortex. With the increase of the number of samples, increase the amount of Buffer viRL and Linear Acrylamide solution in proportion at the same time. If the sample volume is greater than 200μl, the volume of working fluid can be increased in proportion as well.
2. Add 200μl of plasma / serum / cell-free body fluid / cell culture supernatant to the above centrifuge tube (Samples need to equilibrate to room temperature). Vortex for 15 sec and mix thoroughly. To ensure sufficient lysis, the sample and the working solution (Linear Acrylamide) need to be thoroughly mixed
3. Incubate at room temperature (15-25 ℃) for 10min
4. Centrifuge briefly to collect the liquid attached to the tube wall and tube lid
5. Add 350μl of isopropanol, put the tube lid on and vortex for 15sec
6. Centrifuge briefly to collect the liquid attached to the tube wall and lid
7. Carefully transfer 750 μl of the mixture solution from the centrifuge tube to the RNA-Only Column (put RNA-Only Column into collection tube), and put the tube lid on. 8000rpm (~6000 ×g) centrifuge for 1min，discarding the waste liquid in the collection tube
   Note: If flocculent precipitation occurs in the mixture solution, please transfer the precipitate to the RNA-Only Column as well. If the volume of the mixture solution is larger than 750ul, please transfer the mixture twice or multiple times through RNA-Only-Column to completely collect the RNA molecules in the mixture solution and improve the yield
   If the liquid attached on the adsorption column cannot be completely centrifuged into the collection tube, please increase the centrifuge speed and centrifuge time until the liquid has been completely transferred to the collection tube

8. Return the RNA-Only Column to the collection tube, while add all the remaining mixture solution to the RNA-Only Column, and centrifuge at 8000 rpm (~ 6000 × g) for 1 min. Discard the waste liquid in the collection tube

9. Add 500 μl Buffer viRW1 to the RNA-Only Column and centrifuge at 8000 rpm (~ 6000 × g) for 1 min. Discard the waste solution in the collection tube

10. Add 700μl Buffer viRW2 to RNA-Only Column **(Please confirm that you have added anhydrous ethanol before using according to the instructions)**. **8000rpm** (~6000 ×g) centrifuge for 1min, discarding the waste liquid in the collection tube.

11. Repeat step 10.

12. Return the RNA-Only Column to the collection tube. Centrifuge the empty tube at 12,000 rpm (~ 13,400 × g) for 2 minutes. Discard the collection tube.

13. Transfer the RNA-Only Column to a new centrifuge tube. Add 30-50 μl of RNase-Free ddH2O, which has been preheated at 65 ° C, to the center of the RNA-Only Column membrane (Do not add eluent to the clamping ring, otherwise a larger volume of eluent will be lost). Leave it at room temperature for 2min. Centrifuge it at 12,000 rpm (~ 13,400 × g) for 1 min to collect RNA solution
    Note: the volume of RNase-free ddH2O should not be less than 30ul. It might affect the elution efficiency if the volume is too small. The RNA solution after centrifugation can be re-added to RNA-Only-Column, so as to increase the yield of RNA molecules, and repeat the step 13. The RNA solution obtained can be directly used in downstream experiments or stored at -80℃

Determination of RNA purification and concentration

The quality of the obtained RNA is related to many factors during the operation. RNA concentration and purity can be detected by agarose gel electrophoresis and by UV spectrophotometer. As this kit protects the RNA secondary structure well, it is recommended to denature the obtained RNA solution at 72 ℃ for 5-10min before gel electrophoresis

Measure the RNA concentration by using a UV spectrophotometer. An OD260 value of 1 is equivalent to approximately 40 μg / ml of RNA molecules

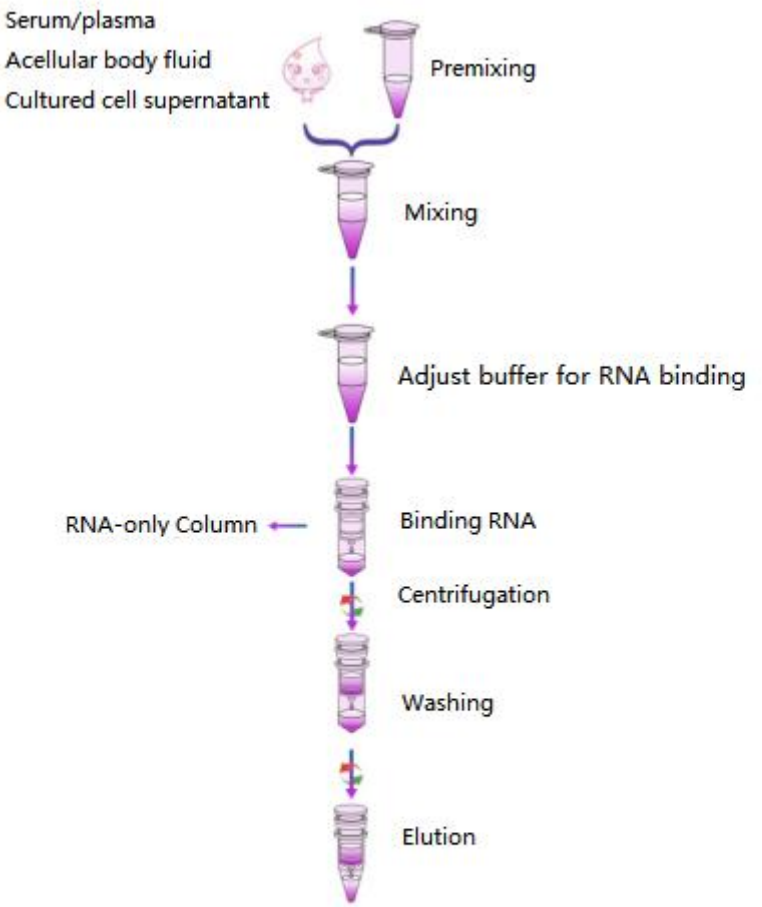
The OD260 / OD280 ratio of RNA is often used as a measure of nucleic acid purity. Generally, the ratio of OD260 / OD280 of pure RNA molecules is 1.8-2.1. The OD260 / OD280 ratio is affected by the pH of the solution used in the assay. For example, OD260 / OD280 readings of purified RNA in 10 mM Tris-HCl buffer at pH 7.5 are between 1.9-2.1. In neutral solutions, the ratio will be lower, which might only be 1.8-2.0, which does not mean that the quality of RNA is getting worse

Detection of DNA contamination

Due to the possible DNA contamination in the sample, the viral RNA isolation kit cannot remove the DNA molecules from the RNA molecules. Its presence is related to the amount of the sample and its nature

For the detection of trace DNA molecules in purified RNA, Real-time PCR detection can be performed without reverse transcription. We suggest that the designed annealing-matched regions of the primers can be located within the introns of the genomic DNA. If the RNA molecule does not contain any genomic DNA fragment, PCR based on this pair of primers will not amplify the corresponding PCR product

Quick-operation diagram



Guides for problems analysis

The following is an analysis of the problems that might be encountered in the extraction of viral RNA. We wish it would be helpful to your experiment. In addition, for other experimental or technical problems other than operating instructions and problem analysis, we have dedicated technical support to help you. Contact us if you need at : 028-83361257or E-mail：Tech@foregene.com。

No RNA can be extracted or the yield of nucleic acid is low

There are usually many factors that affect recovery efficiency, such as: sample RNA content, method of operation, elution volume, etc.。

Analysis of common causes：

1. Ice bath or low-temperature (4 ° C) centrifugation during operation.
   Suggestion: Room temperature (15-25 ° C) operation, never ice bath and low temperature centrifuge.
2. Improper sample storage or sample storage for too long.
   Suggestion: Store samples at -80 ° C or freeze in liquid nitrogen, and avoid repeated freeze-thaw use; try to use freshly collected samples for RNA extraction.
3. Insufficient sample lysis
   Recommendation: Please ensure that the sample and the working solution (Linear Acrylamide) have been thoroughly mixed and incubated for 10 min at room temperature (15-25 ° C)
4. The eluent was added incorrectly
   Recommendation: Make sure that RNase-Free ddH2O is added to the middle of the membrane of the purification column
5. Improper volume of anhydrous ethanol in Buffer viRW2
   Suggestion: Please follow the instructions, add the correct volume of anhydrous ethanol to Buffer viRW2 and mix them well before using the kit。
6. Improper sample usage.
   Suggestion: 200μl of sample per 500μl of Buffer viRL. Excessive sample volume will result in reduced RNA extraction rate.
7. Improper elution volume or incomplete elution.

Suggestion: The eluent volume of the purification column is 30-50μl; if the elution effect is not satisfactory, it is recommended to add pre-heated RNase-Free ddH2O and extend the time placing at room temperature, such as 5-10min

8. Purification column has ethanol residue after rinsing in Buffer viRW2.

Suggestion: If ethanol still remains after rinsing in Buffer viRW2 and empty-tube centrifugation for 2min, the purification column can be left at room temperature for 5min after empty-tube centrifugation to fully remove remaining ethanol.

The degradation of purified RNA molecules

The quality of the purified RNA is related to factors such as sample storage, RNase contamination, and operation.

Analysis of common causes:

1. The collected samples were not saved in time.

Suggestion: If the sample is not used in time after collection, please store it at -80 ℃ or liquid nitrogen immediately. For the extraction of RNA molecules, try to use freshly collected samples whenever possible.

2. Collected samples were freezing and thawing repeatedly.

Suggestion: Avoid repeated freezing and thawing (no more than once) during sample collection and storage, otherwise the yield of nucleic acid will decrease.

3. RNase was introduced in the operating room or no disposable gloves, masks, etc. were worn.

Suggestion: The extraction of RNA molecules experiment is best performed in a separate RNA operation room, and the experimental table is cleaned before the experiment. Wear disposable gloves and masks during the experiment to avoid RNA degradation caused by RNase introduction.

4. The reagent is contaminated by RNase during the use.

Suggestion: Replace with new Viral RNA Isolation Kit for related experiments.

5. The RNase contamination of the centrifuge tubes, pipette tips, etc.

Suggestion: Make sure that the centrifuge tubes, pipette tips, and pipettes are all RNase-Free.

The purified RNA molecules affected downstream experiments

The RNA molecules purified by the purification column will affect downstream experiments if there are too much salt ions or proteins, such as: reverse transcription, Northern Blot, etc.。

1. There are remaining salt ions in the eluted RNA molecules.

   Recommendation: Make sure that the correct volume of anhydrous ethanol has been added to Buffer viRW2, and wash the purification column twice according to the correct centrifugation speed on the operating instructions； If there are still salt ions remaining, you can add Buffer viRW2 to the purification column, and leave it at room temperature for 5min. Then perform centrifugation to remove salt ions contamination to the greatest extent

2. There are remaining ethanol in the eluted RNA molecules

   Suggestion: once confirming that purification columns have been rinsed by Buffer viRW2, perform empty-tube centrifugation according to the centrifugal speed on the operating instructions. If there is still ethanol remaining, it can be left for 5 minutes at room temperature after an empty-tube centrifugation to remove the remaining ethanol to the greatest extent.

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