FOREGENE
EXPERT IN NUCLEIC ACID EXTRACTION

Viral DNA/RNA Isolation Kit

Cat.No.DR-01011/01012/01013

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

For research use only

Store at room temperature

Product Description:

The kit uses the spin column and formula developed by Foregene, which can efficiently extract high-purity and high-quality viral DNA and RNA from samples such as plasma, serum, cell-free body fluid, and cell culture supernatant. The kit specifically adds Linear Acrylamide, which can easily capture small amounts of DNA and RNA from the samples. DNA/RNA-Only Column can efficiently bind DNA and RNA. The kit can process a large number of samples at the same time.

The entire kit does not contain RNase, so the purified RNA will not be degraded. Buffer RW1 and Buffer RW2 can ensure that the obtained viral nucleic acid free of protein, nuclease or other impurities, which can be used directly for downstream molecular biology experiments.

Product Features

◆ The whole process is operated at room temperature (15-25°C), **without ice bath and low temperature centrifugation.**

◆ The complete kit is RNase-Free, so there is no need to worry about RNA degradation.

◆ High nucleic acid yield: DNA/RNA Column and unique formulation can efficiently purify DNA and RNA.

◆ High sample throughput: up to 200μl of sample can be processed at a time.

◆ Fast: Easy to operate and can be done in 20 minutes.

◆ Safe: No organic reagent extraction required.

◆ High quality: The extracted viral nucleic acid is of high purity, free of protein and other impurities, and can meet various downstream experimental applications.

Kit application:

It is suitable for the extraction and purification of viral nucleic acid in samples such as plasma, serum, cell-free body fluid and cell culture supernatant.

Application of Viral Nucleic Acids
Viral DNA/RNA Isolation Kit purified viral DNA and RNA can be used for various downstream molecular experiments, such as: Real Time PCR, RT-PCR, RT-qPCR, molecular cloning, etc.

Storage of nucleic acids
It is recommended to use RNase-Free ddH_2O to elute nucleic acids for immediate use in downstream experiments or to store at -80°C. Nucleic acids can be stored for one year at -80°C.

Product Quality Control
In accordance with FOREGENE's Total Quality Management System, each batch of viral DNA/RNA extraction kits is strictly tested multiple times to ensure the reliability and stability of the quality of each batch of kits.

Kit component

Viral DNA/RNA Isolation Kit			
Kit component	DR-01011	DR-01012	DR-01013
	50 T	100 T	200 T
Linear Acrylamide	120μl	240μl	480μl
Buffer DRL*	25ml	50ml	100ml
Buffer RW1*	25ml	50ml	100ml
Buffer RW2	24ml	48ml	96ml
RNase-Free ddH_2O	10ml	20ml	40ml
DNA/RNA Column	50	100	200
Instructions	1	1	1

* ：Buffer DRL and Buffer RW1 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	~20min (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		

Storage and Stability

- The kit can be stored for 12 months under dry conditions at room temperature (15–25°C); it can be stored at 2–8°C if it needs to be stored for a longer time.

Note: If stored at low temperature, the solution is prone to precipitation. Be sure to place the solution in the kit at room temperature for a period of time before use. If necessary, preheat it in a 37°C water bath for 10 minutes to dissolve the precipitate, and mix well before use.

- Linear Acrylamide solution can be stored for 7 days at room temperature; please take out the kit and store it at -20℃ after receipt.
- After adding Linear Acrylamide to Buffer DRL, it can be stored at 2-8°C for up to 48 hours. Please use it for immediate use

.Kit Component Information

- Linear Acrylamide: Reduce the background binding of the purification column and capture the trace nucleic acids in the system.
- Buffer DRL: Provides the environment required for sample lysis and the environment for passing the column.
- Buffer RW1: Remove impurities such as proteins in nucleic acids.
- Buffer RW2: Remove residual salt ions from nucleic acids.
- RNase-Free ddH_2O: Elute nucleic acids on the purification column membrane.
- DNA/RNA Column: Highly efficient adsorption of nucleic acids.

DNA/RNA Column features

Maximum Nucleic acid binding capacity	20μg
Maximum loading volume	800μl
Minimum elution volume1*	30μl
Selection of samples	Plasma, serum, cell-free body fluid or cell culture supernatant, etc.
Maximum amount of starting material 2*	200μl

1*: The minimum elution volume of 30μl is a reasonable recommended volume given in consideration of nucleic acid recovery rate and concentration. If to increase the yield of nucleic acid, the volume of the eluate can be appropriately increased; if in order to increase the concentration of the purified nucleic acid, a part of the nucleic acid yield is sacrificed,under the premise that the elution volume is appropriately reduced, such as using an elution volume of 15μl, a higher concentration of nucleic acid can be obtained.

2*: For larger sample size, please use multiple DNA/RNA Columns for nucleic acid purification.

Nucleic acid extraction yield and purity

Viral DNA/RNA Isolation Kit can be used to purify viral nucleic acids from samples such as plasma, serum, cell-free body fluids, and cell culture supernatants. The yield is related to the sample itself, initial sample volume, sample freshness, sample storage time and operation related. The purified nucleic acid has OD260/280=1.7-2.1.

Note: (Be sure to read the notes carefully before using)

- Repeated freezing and thawing of the sample should be avoided, otherwise the extracted viral nucleic acid will be degraded and the extraction amount will also decrease.
- The sample processing volume should not exceed 200μl, otherwise the yield and purity of viral nucleic acid will be affected.
- After adding Linear Acrylamide to Buffer DRL, it can be stored at 2-8°C for up to 48h. Please use it now.
- Please add absolute ethanol to Buffer RW2 before using the kit. Please refer to the label on the reagent bottle for the added amount.

  The addition amount of anhydrous ethanol in the kits of different specifications is shown in the following table:

Product specifications	Amount of anhydrous ethanol added
DR-01011	60ml
DR-01012	120ml
DR-01013	240ml

- The nucleic acid yield and quality are related to the elution volume and sample processing volume. It is recommended to use 200 μl of sample volume per 500 μl of Buffer DRL.
- Elution volume: The elution volume should not be less than 30μl, otherwise it will affect the nucleic acid recovery efficiency.
- All experimental steps are carried out at room temperature (15-25℃) unless otherwise specified.

Please check whether there is crystal precipitation in Buffer DRL and Buffer RW1 in the kit. If there is crystal precipitation after storage at low temperature, you can place the Buffer at room temperature or 37°C for a period of time, dissolve the crystal and mix well before use.

Preparation before operation

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

Experimental Materials and Equipment

- 200μl of fresh or frozen plasma, serum, cell-free body fluids and cell culture supernatant
- Benchtop centrifuge (≥13,400×g), pipette, etc.
- Sterile RNase-Free centrifuge tubes, pipette tips, etc.

Self-provided reagents

- Anhydrous ethanol.
- Isopropyl alcohol.

Safety

- This product is for scientific research use only, please do not use it for medicine, clinical medicine, food and cosmetics.
- When using the kit, please wear lab coats, disposable latex gloves, disposable masks, etc. to protect yourself; and avoid artificially introduced RNase contamination to the greatest extent possible.
- Buffer DRL contains chaotropic salts: denaturant, irritant.
- Buffer RW1 contains chaotropic salts: denaturant, irritant.
- Buffer RW2 contains ethanol: Flammable.

Operation guide

The kit is operated at room temperature (15-25°C) throughout the whole process, and ice bath and low temperature centrifugation are not allowed.

Storage of samples

Plasma and serum can be stored at 2-8°C for 6 hours after collection. If long-term storage is required, it can be stored at -20°C or -80°C. Avoid freezing and thawing (not more than once) during storage, otherwise the nucleic acid yield will be reduced. The protein precipitate formed during the freeze-thaw process can block the adsorption column. Therefore, if the protein precipitate is visible to the naked eye after the sample is thawed, centrifuge at 6,800 × g for 3 minutes and carefully aspirate the supernatant for use.

Samples are stored in Buffer DRL

Nucleic acids in Buffer DRL will not be degraded by nucleases. If plasma, serum, cell-free body fluids or cell culture supernatants are added to Buffer DRL for lysis, they can be stored at room temperature for about 24 hours; stored at 4°C for about 1 week ; For longer storage, please store at -80°C, and dissolve the solution at room temperature or 37°C when using.

Initial dosage of sample

The correct initial sample throughput is necessary for optimal nucleic acid yield and purity, and the maximum sample throughput is related to the following factors:

❖ The type of the sample itself and the abundance of nucleic acid in the sample;
❖ The amount of Buffer DRL determines the effective lysis of the sample;
❖ Nucleic acid binding capacity of the DNA/RNA Column.

Based on the above factors, we recommend a sample volume of 200 μl. If the sample processing volume is too large, Buffer DRL will not completely lyse the sample, resulting in low purity of nucleic acid obtained by purification; at the same time, it may exceed the maximum loading capacity of the DNA/RNA Column and waste precious samples.

Material access instructions

Plasma, serum, cell-free body fluids or cell culture supernatant: single treatment, 200μl volume.

Prevent RNase Contamination

◆Human contact is an important source of RNase contamination, please change gloves frequently during operation.

◆Please use RNase-free pipette tips and other plastics.

◆Nucleic acids are not degraded by nucleases while in Buffer DRL, but RNase-free plastic and glassware should be used for further 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, then washed thoroughly with water and autoclaved to remove nucleases.

◆RNase-free water should be used for the preparation of the solution (add the water to the RNase-free glass bottle, add DEPC to a final concentration of 0.01% (v/v), mix well and place overnight and autoclave).

Procedure

Please add absolute ethanol to Buffer RW2 before use. Please refer to the label on the bottle for the added volume.

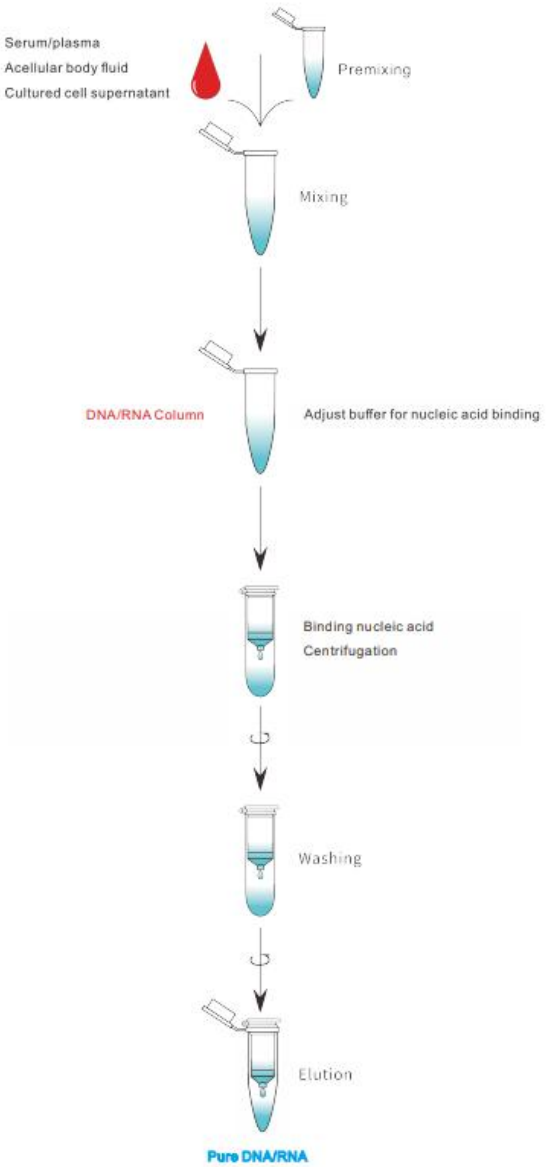
1. Add **500μl** Buffer DRL and **2μl** Linear Acrylamide to an RNase-Free 2ml centrifuge tube, invert back and forth and mix to obtain a lysis working solution.
   Note: Buffer DRL is prone to precipitation at low temperature. If there is precipitation, please incubate it at 37°C to dissolve and mix well before use. To avoid foaming of the solution, do not use a vortex. With the increase of the number of samples, the amount of Buffer DRL and Linear Acrylamide solution was increased in equal proportion.
2. Add **200μl** of plasma (or serum or cell-free body fluid or cell culture supernatant) to the above centrifuge tube (samples need to be equilibrated to room temperature). Vortex for 15 sec to mix.
   Note: To ensure sufficient lysis, the sample and lysis working solution need to be thoroughly mixed.
3. Incubate at room temperature (20-30°C) for 10 minutes, and centrifuge briefly to collect the liquid adhering to the tube wall and cap of the centrifuge tube.
4. Add **350μl** isopropanol, cap the tube and vortex for 15sec to mix thoroughly. Centrifuge briefly to collect the liquid adhering to the tube wall and cap.
5. Carefully transfer **750μl** of the mixture in the centrifuge tube to the DNA/RNA Column (the DNA/RNA Column is placed in the collection tube), cover the tube, centrifuge at 8,000 rpm (~6,000 ×g) for 1 min, and discard the collection tube in the waste liquid.
   Note: If a flocculent precipitate appears in the mixture, transfer the precipitate to the DNA/RNA Column. If the liquid on the adsorption column cannot be completely centrifuged into the collection tube, please increase the rotation speed and prolong the centrifugation time until the liquid is completely transferred into the collection tube.
6. Put the DNA/RNA Column back into the collection tube, add the remaining mixture to the DNA/RNA Column, centrifuge at **8,000 rpm** (~6,000 ×g) for 1 min, and discard the waste liquid in the collection tube.
7. Add **500μl** Buffer RW1 to the DNA/RNA Column, centrifuge at **8,000rpm** (~6,000×g) for 1 min, and discard the waste liquid in the collection tube.
8. Add **700μl** of Buffer RW2 to the DNA/RNA Column (**please confirm that anhydrous ethanol has been added according to the instructions before use**), centrifuge at **8,000rpm** (~6,000×g) for 1min, and discard the waste liquid in the collection tube.
9. Repeat step 8.
10. Put the DNA/RNA Column back into the collection tube, centrifuge the empty tube at 12,000 rpm (~13,400×g) for 2 min, and discard the collection tube.
11. Transfer the DNA/RNA Column to a new centrifuge tube, drop **30-50μl** of pre-warmed RNase-Free ddH_2O at 65°C into the center of the DNA/RNA Column membrane (do not add the eluate to the press ring). Otherwise, a larger volume of eluent will be lost), place at room temperature for 2 min, and centrifuge at 12,000 rpm (~13,400 ×g) for 1 min to collect the DNA/RNA solution.
    Note: The volume of RNase-Free ddH_2O added should not be less than 30μl, and the elution efficiency will be affected if the volume is too small. To improve nucleic acid yield, add the DNA/RNA solution obtained by

centrifugation back to the RNA-only Column and repeat step 11. The resulting DNA/RNA solution can be used directly for downstream experiments or stored at -80°C.

Viral DNA/RNA Analysis

Viral DNA/RNA is present at very low levels in some samples, and we do not recommend using a UV spectrophotometer for concentration and purity testing. PCR, qPCR, RT-qPCR and other methods can be used to determine the virus yield.To analyze the size of viral nucleic acid, please use agarose gel for electrophoresis, followed by virus-specific labeled probes and autoradiography.

Diagram



Problem Analysis Guide

The following is an analysis of the problems that may be encountered in the extraction of viral DNA/RNA, hoping to be helpful to your experiments. In addition, for other experimental or technical problems other than operation instructions and problem analysis, we have dedicated technical support to help you. If you have any needs, please contact us: 028-83360257 or E-mail: Tech@foregene.com.

No nucleic acid extraction or low nucleic acid yield

There are usually many factors that affect the recovery efficiency, such as: sample nucleic acid content, operation method, elution volume, etc.

Analysis of common causes:

1. An ice bath or low temperature (4°C) centrifugation was performed during the procedure.

Suggestion: Operate at room temperature (15-25°C) throughout the whole process, do not ice bath and low temperature centrifugation.

2. The sample was stored improperly or the sample was stored for too long.

Recommendation: Store samples at -80°C and avoid repeated freezing and thawing; try to use freshly collected samples for nucleic acid extraction.

3. Insufficient sample lysis.

Recommendation: Please ensure that the sample and lysis working solution are thoroughly mixed and incubated at room temperature (15-25°C) for 10 minutes.

4. Incorrect addition of eluent.

Suggestion: Make sure that RNase-Free ddH2O is added dropwise to the middle of the purification column membrane, and do not drop it on the purification column ring.

5. The correct volume of absolute ethanol was not added to Buffer RW2.

Suggestion: Please follow the instructions, add the correct volume of absolute ethanol to Buffer RW2 and mix well before using the kit.

6. Inappropriate sample volume.

Suggestion: 200μl of sample is processed for every 500μl of Buffer DRL. Excessive sample processing will result in lower nucleic acid extraction yield.

7. Inappropriate elution volume or incomplete elution.

Recommendation: The eluent volume of the purification column is 30-50μl; if the elution effect is not satisfactory, it is recommended to extend the time at room temperature after adding preheated RNase-Free ddH2O, such as 5-10min.

8. Ethanol remains on the column after washing with Buffer RW2.

Suggestion: If ethanol remains after centrifugation with Buffer RW2 for 2 minutes, the column can be placed at room temperature for 5 minutes after centrifugation to fully remove residual ethanol.

The purified nucleic acid is degraded

The quality of the purified nucleic acid is related to the preservation of the sample, RNase contamination, operation and other factors. Analysis of common causes:

1. The collected samples were not stored in time.

Suggestion: If the sample is not used in time after collection, please store it at -80°C at low temperature immediately. For RNA extraction, try to use freshly collected samples.

2. Collect samples and freeze and thaw repeatedly.

Suggestion: Avoid freezing and thawing (not more than once) during the collection and storage of samples, otherwise the nucleic acid yield will be reduced.

3. RNase is introduced in the operation room or disposable gloves, masks, etc. are not worn.

Recommendation: RNA extraction experiments are best performed in a separate RNA operation room, and the laboratory table should be cleaned before the experiment.

Wear disposable gloves and masks during the experiment to avoid RNA degradation caused by the introduction of RNase to the greatest extent.

4. The reagent was contaminated with RNase during use.

Recommendation: Replace with a new Viral DNA/RNA Isolation Kit for related experiments.
5. The centrifuge tubes and pipette tips used for RNA manipulation are contaminated with RNase.
Suggestion: Make sure that the centrifuge tubes, pipette tips, pipettes, etc. used for RNA extraction are all RNase-Free.

Purified nucleic acid affects downstream experiments

DNA and RNA purified by the purification column, if the salt ion and protein content are too high, it will affect the downstream experiments, such as: PCR amplification, reverse transcription, etc.
1. The eluted DNA and RNA have residual salt ions.
Suggestion: Make sure that the correct volume of absolute ethanol is added to Buffer RW2, and wash the purification column twice at the centrifugation speed specified in the operating instructions; Perform centrifugation to minimize salt ion contamination.
2. The eluted DNA and RNA have ethanol residues.
Suggestion: After confirming the washing with Buffer RW2, perform empty tube centrifugation at the centrifugation speed in the operating instructions; if there is still ethanol residue, you can centrifuge the empty tube and then place it at room temperature for 5 minutes to remove the ethanol residue to the greatest extent.

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