ROJETECHNOLOGIES

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DNJia Virus DNA Kit

Viral DNA/RNA isolation based on silica technology

- MiniPrep

For Viral DNA Isolation from

Body Fluid
Serum
Plasma

By ROJE

Edition, 12/2022

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
GLB	20ml
BWB1 (concentrate)	2 x 16ml
BWB2 (concentrate)	2 x 12ml
ERR	5ml
RJ-Protease	2 x 1.25ml
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. We suggest storing RJ-Protease at 2-8°C, and for routine use, it is recommended that you aliquot it to 100µl volumes and storage at 2-8°C. When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

DNJia Virus DNA Kit provides the components and procedures necessary for purifying viral DNA from cell-free samples such as body fluid, serum, plasma and animal tissue. Notice that, DNJia Virus DNA Kit is intended for molecular biology applications not for diagnostic use. We recommend all users to study DNA 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

DNall VirAll 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 Cat and Lot number on web at www.rojetechnoloes.com.

Description

The DNJia Virus DNA Kit is designed for rapid and efficient purification of high-quality viral DNA 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 for nucleic acid isolation. DNJia Virus DNA Kit needs less handling and it is convenient for simultaneous isolation, which makes it favorites for laboratory with many isolations in a day. The purified viral nucleic acids are free of proteins, nucleases, and other contaminants or inhibitors of downstream applications. Isolated DNA can be directly used in PCR, qPCR or other nucleic acid-based assays.

Procedure

DNJia Virus DNA Kit is designed for isolating both viral DNA from body fluid, serum, plasma etc. Lysis is achieved by incubation of the sample in GLB; and in a RJ protease enzyme solution. Appropriate conditions for DNA binding to the silica membrane is achieved by the addition of ethanol to the lysate. Then, DNA is selectively bound to the membrane. Contaminants are removed by two specific washing buffers. Pure viral DNA is finally eluted in

rehydration buffer. Isolated DNA 6 is ready to use in downstream applications. A symmetric peak at 260 nm by spectrophotometer, confirms high purity of isolated nucleic acid.

Equipment & Reagents to Be Supplied by User

- Molecular biology grade 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 DNA/RNA can be used in many downstream applications:

- Different kinds of PCRs
- Viral genotyping
- Viral detection
- Viral load monitoring

Features

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

Table 1: DNJia Virus DNA Kit features and specifications

Features	Specifications
Elution volume	20-200µl
Technology	Silica technology
Main sample type	Body Fluid, serum, Plasma
Processing	Manual
Sample amount	Plasma and serum: Up to 200 µl
Biomolecule isolation	DNA
Operation time per reaction	Less than 30min
Typical yield	Varies
Enzyme	RJ-Protease

Sample Preparation

- If possible, use only fresh sample material. Do not freeze/thaw samples more than once.
- Plasma and serum samples can be stored at 2-8°C for up to 24 hours, or at −20°C or −70°C for long-term storage.
- Before use, equilibrate samples to room temperature (20±5°C). Remove precipitates from plasma/serum samples, if any, by centrifugation for 5min at 3,000 × g.
- Use EDTA or citrate treated plasma samples.
- Urogenital swabs can be stored at 2-8°C for up to 48 hours. For longer term storage cells should be collected by centrifugation and stored at −20°C or −70°C
- Nasal and buccal swabs can be stored at 2-8°C for up to 48 hours.

Washing Buffer Preparation

Before the first use, add appropriate amount of ethanol (%96-100) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 2. 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 2: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
BWB1	16ml	24ml	40ml
BWB2	12ml	28ml	40ml

Protocol 1, Isolation of Viral DNA

Sample type:

- Body fluid
- Serum
- Plasma

Recommended Starting Material for Isolation of Viral DNA

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

Table 3: Recommended starting material and Lysis Buffer amount

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

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Do not forget to add the appropriate amount of molecular biology grade ethanol (%96–100) to BWB1 and BWB2 buffers as indicated on the bottle, before using for the first time, refer to washing buffer preparation.
- If GLB or TLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

- Add 25µl RJ-Protease to a 1.5ml clean microcentrifuge tube. Add 200µl sample (plasma, serum, body fluid and etc.) to the tube. Then add 200µl GLB. Pulse vortex for 15 s and incubate at 56°C for 12min.
- Add 200µl ethanol (%96-100) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.
- Gently, pipette the mixture to a HiPure DR column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 8000 rpm for 1min. Discard flow-through and place back the HiPure DR column in to the collection tube.
- Add 500µl BWB1 and centrifuge for 1min at 8000 rpm, discard both the flow-through and the collection tube. Place back the HiPure DR column in to the collection tube.
- Add 500µl BWB2 and centrifuge for 3min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR column in a new clean 1.5ml microcentrifuge tube (not provided).

  Note: To avoid ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1min at 14000 rpm.
- Pipette 30-50µl ERR directly onto HiPure DR column. Incubate at room temperature for 1-5min. Centrifuge it at 12000 rpm for 1min.

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 DNA Isolation	**Low DNA yield**	• Insufficient lysis	• Please refer to Table 3 to apply best match for size of starting material and amount of lysis buffer.
			• Make sure to do pulse-vortexing after addition of lysis buffer and RJ-Protease.
		• Too few viruses in the sample	• Do the test with new samples.
		• Incomplete lysing	• Repeat the reaction once more and make sure to mix the sample and lysis buffer completely by pulse-vortexing.
		• Reagents not applied correctly	• Prepare buffers according to the protocol.
			• Make sure ethanol is added to BWB1 and BWB2.
			• Repeat the procedure with a new sample.
		• DNA improperly eluted	• The best buffer for DNA rehydration is prepared in the Kit Box. We insist to use the supplied rehydration buffer, however if you want to use water instead, make sure that the pH is at least 7.0, or use 10 mM Tris-HCl Ph≥ 7.0.
	DNA does not perform well in downstream applications	• DNA was not washed with the provided washing buffer	• Ensure the column was washed once with prepared BWB1 and once more with prepared BWB2, respectively.

		• Ethanol carryover	• Ensure that the traces of ethanol before rehydration step is removed.
General Handling	**Column clogging**	Precipitates were not removed.	• When using plasma samples, remove visible Cryoprecipitates by centrifugation for 5min at 3000 × g
		Lysate not completely passed through the membrane	• Centrifuge for 1min at full speed or until all the lysate has passed through the membrane.

Ordering Information

Category	Product name	Cat NO.	Size
DNA Technologies	DNJia Virus DNA Kit	DN983056	100 preps
	DNall VirAll Kit	DN983053	100 preps
	RNJia Virus Kit (without BFC)-	RN983040	100 Preps
	DNall Plus Kit	DN983049	100 preps
	DNJia Plus Blood and Cell Kit	DN983046	100 preps
	DNSol, MiniPrep	DN983003	100 preps
	DNSol, MidiPrep	DN983014	50 preps
	DNSol, MaxiPrep	DN983018	50 preps
	DNSol Clotted Blood Kit	DN983032	50 preps
	DNJia AmnioPure Kit	DN983045	100 preps
	Hashin	LD983003	2ml
Related Product	RJ-Protease, Recombinant (20mg/ml)	EB983121	1ml

Technical Assistance

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

NO. 2 Farvardin street- Fernan Street- Tehran- Shahr Qods- Iran- Postal Code: 37531146130- phone: +982191070705

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