ROJETECHNOLOGIES

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DNJia CF Kit

Free circulating nucleic acid isolation based on silica technology

For free-circulating DNA, RNA, miRNA, and viral nucleic acids

from

Serum
Plasma
Urine
Cell-free body fluids

By ROJE Edition: 11/2020

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	50 preps
CFL	2 x 60 ml
CFB (concentrate)	5 x 36 ml
ERW1 (concentrate)	19 ml
ERW2 (concentrate)	13 ml
ERR	10 ml

Carrier RNA	620 µg
CF-Protease	2*8.5 ml
CF- Column	50
Collector	50
Connector	50
Collection Tube	50

Storage

Shipment condition is checked by ROJETechnologies. After arrival, all reagents should be kept dry, at room temperature. We suggested storing CF-Protease at -20 °C for longer stability; However, for routine use it could be stored 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 CF Kit provides the components and procedures necessary for purifying cell free DNA or RNA from serum or plasma. 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

DNJia CF 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

DNJia CF Kit provides a rapid, careful and convenient method for high quality cell free DNA or RNA isolation from Serum and plasma. This kit is based on silica column technology for isolation of concentrated, highly purified, intact genomic DNA which is suitable to use for variety of downstream processes such as PCR analysis, NIPT and Sex determination test.

Procedure

DNJia CF Kit is designed for isolating cell free nucleic acid from serum and plasma. Lysis is achieved by incubation of the sample in a CF-Protease enzyme solution and ROJE specific lysis buffer. Appropriate conditions for DNA binding to the silica membrane is achieved by the addition of chaotropic salts and ethanol to the lysate, then DNA is selectively bound to the membrane. Contaminants are removed by two specific washing buffers. Pure nucleic acid is finally eluted in rehydration buffer. Isolated nucleic acid is ready to use in downstream applications. Purified nucleic acids are free of impurities such as proteins and nucleases.

Equipment & Reagents to Be Supplied by User

- Ethanol (96-100%)
- Isopropanol
- Pipets and pipet tips
- 1.5 ml Microtube
- Vortex
- Dry Heat Block/ Water Bath
- Centrifuge
- Vacuum device

Applications

The isolated nucleic acid can be used in many downstream applications:

- PCR analysis
- NIPT
- Sex determination test

Features

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

Table 1. DNJia CF Kit features and specifications

Features	Specifications
Elution volume	20-150 µl
Technology	Silica technology
Main sample type	Serum/ plasma/ urine / cell-free body fluids
Processing	Manual
Sample amount	Up to 3 ml
Operation time per reaction	Less than 2 h
Typical yield	Varies
Average purity	A260/A280= 1.7-2.0
Size of nucleic acid purified	Less than 1000 bp (DNA)
Enzyme	CF-Protease
Carrier RNA	Provided in the kit

Recommended Starting Material

To reach optimized results it is better to follow as listed here. The size of recommended material is written in Table 2.

Table 2: Appropriate size of starting material

Sample	Size of Starting Material
Serum/ plasma/ urine / cell-free body fluids	Up to 3 ml

Plasma Preparation and storage

- 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.
- Use EDTA or citrate treated plasma samples.
- Before use, equilibrate samples to room temperature (20±5°C).

Process

For isolating cell-free circulating DNA and to remove cellular debris and thus reduces the amount of cellular or genomic DNA and RNA in the sample use this protocol for sample preparation:

1. Add blood into EDTA-tube and centrifuge it for 10 min at 1900 x g (3000 rpm) and 4°C temperature.
2. Carefully remove plasma supernatant without disturbing the buffy coat layer.
   Note: About 4–5 ml plasma can be obtained from one 10 ml primary blood tube.

Now you can use Plasma for circulating nucleic acid isolation at this stage.

For removing additional cellular debris and contamination continue the process:

3. Transfer plasma into fresh 15 ml centrifuge tubes. Centrifuge plasma samples for 10 min at 16,000 x g and 4°C temperature.
   Note: This will remove additional cellular nucleic acids attached to cell debris. Carefully remove supernatant to a new tube with a pipette without disturbing the pellet.

Note: If plasma will be used for nucleic acid isolation on the same day, store at 2–8°C. For longer storage, keep plasma frozen at –80°C. Before using the plasma for circulating nucleic acid isolation, thaw plasma tubes at room temperature.

In circumstances using cryoprecipitates, follow these 2 steps for preparing plasma sample:

A. To remove cryoprecipitates, centrifuge plasma sample for 5 min at 16,000 x g and 4°C temperature.
B. Transfer supernatant to new tube and begin with nucleic acid isolation protocol.

Carrier RNA Preparation

Add 620 µl ERR to the tube containing 620 µg lyophilized carrier RNA to obtain a solution of 1 µg/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 CFL. It must first be dissolved in ERR and then added to CFL.

CFL Preparation

Calculate the volume of Buffer CFL–carrier RNA mix needed per batch of samples to be simultaneously processed from Table 3.

Table 3. Volumes of Buffer CFL and carrier RNA (dissolved in Buffer ERR) required for processing 1 ml, 2 ml, or 3 ml samples

Number of samples	Buffer CFL (ml)			Carrier RNA in Buffer ERR (µl)
1	0.9	1.8	2.6	11.2
2	1.8	3.5	5.3	22.6
3	2.6	5.3	7.9	33.8
4	3.5	7.0	10.6	45
5	4.4	8.8	13.2	56.2
6	5.3	10.6	15.8	67.6
7	6.2	12.3	18.5	78.8
8	7.0	14.1	21.1	90
9	7.9	15.8	23.8	101.2
10	8.8	17.6	26.4	112.6
11	9.7	19.4	29.0	123.8
12	10.6	21.1	31.7	135
13	11.4	22.9	34.3	146.2
14	12.3	24.6	37.0	157.6
15	13.2	26.4	39.6	168.8
16	14.1	28.2	42.2	180
17	15.0	29.9	44.9	191.2
18	15.8	31.7	47.5	202.6
19	16.7	33.4	50.2	213.8
20	17.6	35.2	52.8	225
21	18.5	37.0	55.4	236.2
22	19.4	38.7	58.1	247.6
23	20.2	40.5	60.7	258.8
24	21.1	42.5	63.4	270

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

Note: The sample-preparation procedure is optimized for 5.6 µg 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 CFL.

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 4. 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 4: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
ERW1	19 ml	25 ml	44 ml
ERW2	14 ml	32 ml	46 ml

CFB Preparation

Before the first use, add appropriate amount of isopropanol to CFB tube, then mix thoroughly to prepare CFB buffer, refer to Table 5.

Table 5: CFB buffer preparation

Buffer Name	Concentrated Volume	Amount of Isopropanol	Final Volume
CFB	36 ml	24 ml	60 ml

Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Samples

Sample Type: Serum, Plasma, Urine

Some tips to know

- Green denotes sample volumes of 1 ml serum, plasma or urine; blue denotes sample volumes of 2 ml serum, plasma or urine; red denotes sample volumes of 3 ml serum, plasma or urine.
- All centrifugation steps are carried out at room temperature (15–25°C).
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.
- Equilibrate samples to room temperature.
- If samples are less than 1 ml, 2 ml, or 3 ml, bring the volumes up to 1 ml, 2 ml, or 3 ml with phosphate buffered saline.
- Set up your vacuum device.
- Adjust a heating block to 60°C for use with 50 ml centrifuge tubes in step 4.
- Adjust a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate ERR to room temperature for elution in step 15.
- Add carrier RNA reconstituted in ERR to CFL refer to buffer preparation part
- Add appropriate amount of 96-100 ethanol to ERW1 and ERW2, refer to buffer preparation part.
- Add appropriate amount of isopropanol to the CFB buffer according to their label, refer to buffer preparation part
- If CFL forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

1. Pipet 100 µl, 200 µl, or 300 µl CF-Protease into a 50 ml microtube (not provided).
2. Add 1 ml, 2 ml, or 3 ml of serum, plasma or urine to the 50 ml tube.
3. Add 0.8 ml, 1.6 ml, or 2.4 ml prepared CFL (refer to CFL preparation part) then, pulse-vortexing for 30 s.
   Make sure that a visible vortex forms in the tube. In order to ensure efficient lysis, it is essential that the sample and CFL are mixed thoroughly to yield a homogeneous solution.
   Note: Proceed immediately to step 4 to start the lysis incubation.
4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add 1.8 ml, 3.6 ml, or 5.4 ml CFB to the lysate in the tube. Then, pulse vortexing for 15–30 s.
7. Incubate the lysate–CFB mixture for 5 min on ice.
8. Insert the CF-Column into the Connector on the vacuum device. Insert a 20 ml tube extender into the open CF-Column or continuously add the lysate to the CF-Column until it is passed completely.
   Make sure that the tube extender is firmly inserted into the CF-Column in order to avoid leakage of the sample.
   Note: Keep the collection tube for the dry spin in step 13.
9. Carefully transfer the lysate–CFB mixture from step 7 into the tube extender of the CF-Column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.
   Please note that large sample lysate volumes (about 11 ml when starting with 3 ml sample) may need up to 10 minutes to pass through the membrane by vacuum force. For fast and convenient release of the vacuum pressure, the vacuum regulator should be used (part of the vac connecting system).
   Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring CF-Columns.
10. Add 600 µl ERW1 to the CF-Column. Leave the lid of the column open, and switch on the vacuum pump. After all of ERW1 has been drawn through

    the CF-Column, switch off the vacuum pump, and release the pressure to 0 mbar.

11. Add 750 µl ERW2 to the CF-Column. Leave the lid of the column open, and switch on the vacuum pump. After all of ERW2 has been drawn through the CF-Column, switch off the vacuum pump and release the pressure to 0 mbar.
12. Add 750 µl of ethanol (96–100%) to the CF-Column. Leave the lid of the column open, and switch on the vacuum pump. After all of the ethanol has been drawn through the CF-Column, switch off the vacuum pump and release the pressure to 0 mbar.
13. Close the lid of the CF-Column. Remove it from the vacuum manifold, and discard the Connector. Place the CF-Column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
14. Place the CF-Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.
15. Discard the 2 ml collection tube from step 14. Place the CF-Column in a clean 1.5 ml microtube. Carefully add 20–150 µl of ERR into the center of CF-Column. Incubate at room temperature for 3 min.

    Important: Equilibrate ERR to room temperature (15–25°C). Elution volume is flexible and can be adapted according to the requirements of downstream applications.
16. Centrifuge the microtube at full speed (20,000 x g; 14,000 rpm) for 1 min.

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

Low nucleic acid yield	Inappropriate sample storage condition	• Do not freezing and thawing samples more than once. Repeat the purification procedure with new samples. • Do not left the sample for long time at room temperature. Repeat the purification procedure with new samples.
	Diluted target nucleic acid	• Decrease time between blood draw and plasma preparation.
	Carrier RNA not added to CFL	• Reconstitute carrier RNA in ERR and mix with CFL as described before. Repeat the purification process with new samples.
	Carrier RNA is degraded	• After reconstitution in ERR, not stored at -20˚C. • Multiple freeze–thaw cycles. In each case, reconstitute RNA carrier in ERR again and prepared new CFL, then repeat the procedure
	Insufficient lysis	• Make sure to do pulse-vortexing vigorously after addition of lysis buffer and CF-Protease.
		• Incubate mixture of the sample and lysis buffer for an additional 15-20 min at 60° C.
		• Ensure mixing sample completely before incubation step.

	Too few cells in the sample	• Do the test with new samples.
	Reagents not applied correctly	• Prepare buffers according to the buffer preparation part. • Make sure ethanol is added to ERW1 and ERW2. • Repeat the procedure with a new sample. • Make sure isopropanol was added to CFB. Refer to CFB preparation part.
	DNA elution is incomplete	• Perform rehydration step once more, by adding another 20-150 µl ERR to column and incubate at room temperature before centrifugation. • Check that all previous steps are done appropriately.
	Nucleic acid improperly eluted	• The best buffer for nucleic acid rehydration is prepared in the Kit Box. We insist on using 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.
Nucleic acids do not perform well in downstream applications	PCR reaction condition is not optimized	Make sure that PCR condition is optimized by testing: • Primer designs and annealing conditions • Changing source of Taq Polymerase • Different amount of DNA sample

	Nucleic acid was not washed with the provided washing buffer	• Ensure the column was washed once with prepared ERW1 and once more with prepared ERW2, respectively.
	Ethanol carryover	• Preform another centrifugation before rehydration step to ensure no remaining of ethanol on column.
	Do not use standard buffer for rehydration	• Use ROJE rehydration buffer for dissolving purified nucleic acid.
	Carrier RNA interfering	• reduce the amount of carrier RNA.
Clogged Column	Cryoprecipitates may have formed in plasma	• Do not freeze and thawed plasma sample more than once. These can block the column. If it happens, clear the sample by centrifugation for 5 min at 16,000 x g.
	Vacuum pressure of 800–900 mbar not reached.	• The vacuum manifold is not tightly closed. Press down on the lid of the vacuum manifold after the vacuum is switched on. Check if vacuum pressure is reached. • Check the seal of the manifold visually and replace it if necessary. • Connection to vacuum pump is leaky. Exchange the connections between pump and vacuum manifold if necessary.

Ordering Information

Category	Product name	Cat NO.	Size
DNA Technologies	DNJia CF Kit	DN983054	50 preps
	DNJia CF Micro Kit	DN983055	50 preps
	DNJia CF Micro Kit	DN983061	100 Preps

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 available at ROJETechnologies website.
- 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

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