ROJETECHNOLOGIES

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Handbook

DNJia Micro Kit

DNA and Cell Free isolation based on silica technology

- MiniPrep

For DNA Isolation from

Amniotic Fluid

Semen

For Cell-Free DNA Isolation from

Blood
Serum
Plasma
Cells
Body fluid
Semen

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.

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Contents

Kit Content

Component	100 preps
GLB	60 ml
BWB1 (concentrate)	2 X 16 ml
BWB2 (concentrate)	2 X 16.5 ml
RRB	20 ml
RJ-Protease	4 x 1.5 ml
Micro Column	100
Collection Tube	300
Carrier RNA	620 µg

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. Also, Carrier RNA is storable at room temperature before preparation. However, after adding RRB 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

DNJia Micro Kit provides a rapid, careful, convenient and phenol-free method for high quality genomic and Cell Free DNA isolation from body fluid and amniotic fluid. The procedure is based on spin column technology, which takes less than 30 minutes. DNJia Micro Kit needs less handling and it is convenient for simultaneous isolation, which makes it favorites for laboratory with many isolations in a day. Typical yields of genomic DNA vary, depending on cell density of samples. The isolated DNA is ready to use in downstream applications such as PCR and rt-PCR analysis.

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 Micro 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 Micro Kit provides a rapid, careful, convenient and phenol-free method for high quality genomic and Cell Free DNA isolation from body fluid and amniotic fluid. The procedure is based on spin column technology, which takes less than 30 minutes. DNJia Micro Kit needs less handling and it is convenient for simultaneous isolation, which makes it favorites for laboratory with many isolations in a day. Typical yields of genomic DNA vary, depending on cell density of samples. The isolated DNA is ready to use in downstream applications such as PCR analysis and restriction endonuclease digestions.

Procedure

DNJia Micro Kit is designed for isolating DNA from 600µl-6ml body fluid and amniotic fluid. Lysis is achieved by incubation of the sample in a RJ-Protease enzyme solution and GLB. 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 specifics washing buffers. Pure DNA is finally eluted in rehydration buffer. Isolated DNA is ready to use in downstream applications. It has A 260/A 280 ratios of 1.7-2, and a symmetric peak at 260 nm by spectrophotometer, confirms high purity.

Equipment & Reagents to Be Supplied by User

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

Applications

The isolated DNA can be used in many downstream applications:

- Different kinds of PCRs, including Long-range PCR
- Sequencing
- Restriction digestion
- Southern blotting

Features

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

Table 1. DNJia AmnioPure Kit features and specifications

Features	Specifications
Elution volume	25-50 µl
Technology	Silica technology
Main sample type	Amniotic fluid-body fluid
Processing	Manual
Sample amount	Up to 600 µl- 6ml
Operation time per reaction	Less than 30 Min
Typical yield	Varies
Average purity	A260/A280= 1.7-2.0
Size of DNA purified	Up to 50 Kb
Enzyme	RJ-Protease

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 including:

Sample	Size of Starting Material	Lysis Buffer Amount
Amniotic fluid	5-6 ml	350 µl
Blood, plasma, serum or body fluid	600 µl	600-700 µl
Sperm	300 µl	480 µl

Before Start

- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and will not influence the efficiency of buffer.

- Not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation.

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

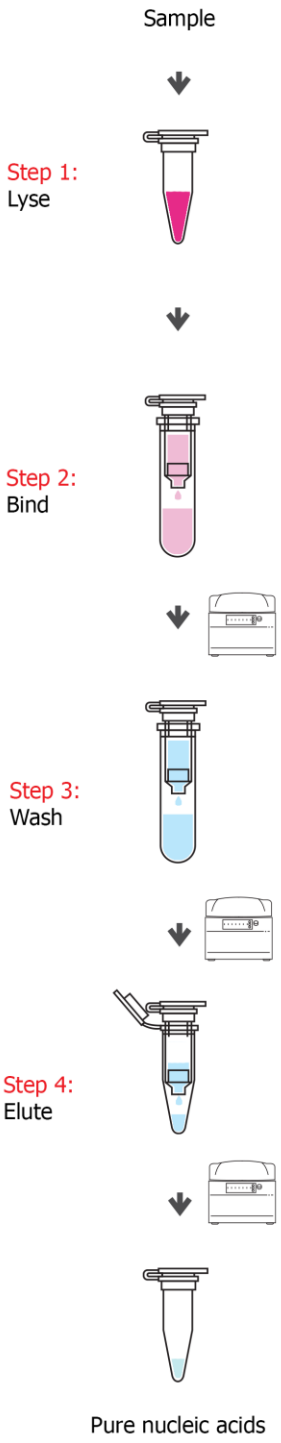
Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
BWB1	16 ml	24 ml	40 ml
BWB2	16.5 ml	38.5 ml	55 ml

Maximize DNA Yield

To obtain higher yield of DNA, it is important to follow protocol carefully and pay attention to sample size table (refer to table 2), which is recommended for samples. It is good to know that:

- Avoid freezing and thawing samples, which may result in decreased DNA yield and size, compared to fresh samples.
- We suggested storing RJ-Protease at -20°C for longer stability; However, for routine use, it is recommended that RJ-Protease aliquoted to 100µl volumes before storage at 2-8°C and avoid frequent freeze-thaw.
- Carrier RNA is storable at room temperature before preparation. However, after adding RRB buffer, it is recommended that it must store at -20°C and be aliquoted to 100µl volumes and avoid frequent freeze-thaw.

Procedure of silica-based DNA isolation in quick look



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 ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time.
- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Protocol 1

- ***Isolation of Genomic DNA from Amniotic fluid (based on silica technology)***

Process

1. Centrifuge 1.5 ml of the amniotic fluid for 10 min at 5000 rpm in a clean microcentrifuge tube, discard the supernatant and repeat this step until you pellet 5-6 ml amniotic fluid.
2. Add 350 µl GLB to the pellet then 35 µl RJ-Protease. Pulse vortex until the pellet is dissolved thoroughly incubate at 56 °C for 20 min, pulse vortex for 15 s every 6 min during the incubation.
3. Add 350 µl ethanol (96-100%) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.
4. Gently, pipette the mixture to a Micro Column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 8000 rpm for 1 min. Discard flow-through and place back the Micro Column in to the collection tube.
5. Add 650 µl BWB1 and centrifuge for 1 min at 8000 rpm, discard both the flow-through and the collection tube. Place back the Micro Column in to the collection tube.
6. Add 750 µl BWB2 and centrifuge for 15 s at 10000 rpm. discard both the flow-through and the collection tube. Place back the Micro Column in to the collection tube.
7. Add 500 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the Micro Column in a new clean 1.5 ml 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 1 min at 14000 rpm.

8. Pipette 25-50 µl RRB directly onto Micro Column. Incubate at room temperature for 5 min. Centrifuge it at 12000 rpm for 2 min.

Protocol 2

- ***Isolation of genomic and Cell-Free DNA from semen***

A) Isolation of Genomic DNA from semen

Sample Type: Semen

Some tips to know:

- All steps, before applying the sample to Micro Column, are carried out on the ice. Before using the protocol for the first time, add 2-Mercaptoethanol to GLB, 4% of GLB volume. It is better to calculate it every time you test and add 4% of 2ME to it for higher efficiency. It is 480 µl of GLB and 20 µl of 2ME for each sample test. 2-Mercaptoethanol is commercially available and due to safety issues not provided in the kit.
- Before using the protocol for the first time, add isopropanol to TLB, 40% of TLB volume. It is better to calculate it every time you test and add 40% of isopropanol to it for higher efficiency. It is 300 µl of TLB and 200 µl of isopropanol for each sample test. Isopropanol is commercially available and due to safety issues not provided in the kit.

Attention: It is recommended to prepare it freshly. It is not stable for more than 2 weeks.

- If TLB & GLB forms a precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage conditions and will not influence the efficiency of the buffer
- Do Not forget to add the appropriate amount of ethanol (molecular biology grade, 96–100%) to BWB1 and BWB2 as indicated on the bottle, before using them for the first time. Refer to the washing buffer preparation section.

Process

1.Add 75 µl RJ-Protease to a 1.5 ml clean microcentrifuge tube. Add 300 µl semen to the tube. Then add 500 µl GLB (prepared 2-ME). Pulse vortex for 1 min and incubate at 60 °C for 2h. Every 10 minutes invert it 20 times Pulse vortex for 3s.

2.Add 500 µl TLB (prepared isopropanol). Pulse vortex for 20s and incubate at 4 °C for 3min then incubate at 70°C for 1min. Transfer up to 700 µl of the sample, including any

precipitate that may have formed, to a Micro Column placed in a 2ml collection tube (supplied in the kit box). Close the lid gently, and centrifuge for 1min at 8000 rpm. Discard the flow-through. Reuse the collection tube in step 10

3.Add the remainder of the sample to the column and centrifuge for 1min at 8000 rpm Discard the flow-through new collection.

4.Add 650 µl BWB1 to the Micro Column. Centrifuge for 1min at 8000 rpm at room temperature. Discard the flow-through.

5.Add 750 µl BWB2 to the Micro Column. Centrifuge for 3min at 14000 rpm at room temperature. Discard the flow-through new collection Centrifuge for 1 min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.

6.Place the Micro Column in a new 1.5 ml microtube. Add 50µl RRB directly to the Micro Column membrane and incubation in RT for 3-5min then Centrifuge for 75s at 13000 rpm.

B) Isolation of Cell Free DNA from semen

Sample Type: Semen

Some tips to know:

- All steps, before applying the sample to Micro Column, are carried out on the ice. Before using the protocol for the first time, add 2-Mercaptoethanol to GLB, 4% of GLB volume. It is better to calculate it every time you test and add 4% of 2ME to it for higher efficiency. It is 480 µl of GLB and 20 µl of 2ME for each sample test. 2-Mercaptoethanol is commercially available and due to safety issues not provided in the kit.
- Before using the protocol for the first time, add isopropanol to TLB, 40% of TLB volume. It is better to calculate it every time you test and add 40% of isopropanol to it for higher efficiency. It is 300 µl of TLB and 200 µl of isopropanol for each sample test. Isopropanol is commercially available and due to safety issues not provided in the kit.

Attention: It is recommended to prepare it freshly. It is not stable for more than 2 weeks.

- If TLB & GLB forms a precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage conditions and will not influence the efficiency of the buffer

- Do Not forget to add the appropriate amount of ethanol (molecular biology grade, 96–100%) to BWB1 and BWB2 as indicated on the bottle, before using them for the first time. Refer to the washing buffer preparation section.

Process

1. 500 microliters of sperm sample into a vial and put in a centrifuge at 900 × g for 10 minutes. Then, transfer the supernatant to the sterile 1.5 vials.
2. Add 90 µl RJ-Protease and 15 µl RNA carriers. Then add 500 µl GLB (prepared 2-ME). Pulse vortex for 30s and incubate at 60 °C for 1h. Every 10 minutes invert it 20 times Pulse vortex for 3s.
3. Add 400 µl TLB (prepared isopropanol). Pulse vortex for 20s and incubate at 4 °C for 5min then incubate at 70°C for 1min. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to a Micro Column placed in a 2ml collection tube (supplied in the kit box). Close the lid gently, and centrifuge for 30min at 12000 rpm. Discard the flow-through. Reuse the collection tube.
4. add the remainder of the sample to the column and centrifuge for 30s at 12000 rpm Discard the flow-through new collection.
5. Add 650 µl BWB1 to the Micro Column. Centrifuge for 30s at 12000 rpm at room temperature. Discard the flow-through.
6. Add 750 µl BWB2 to the Micro Column. Centrifuge for 3min at 14000 rpm at room temperature. Discard the flow-through new collection Centrifuge for 1 min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.
7. Place the Micro Column in a new 1.5 ml microtube. Add 30µl RRB directly to the Micro Column membrane and incubation in RT for 5min then Centrifuge for 2min at 14000 rpm.

Protocol 3

- **Isolation of Cell Free DNA (based on silica technology)**

Sample type:

- Animal blood
- Cells
- Body fluid
- Serum
- Plasma

Process

1. Add 60 µl RJ-Protease to a 2 ml clean microcentrifuge tube. Add 600 µl blood (plasma, serum or body fluid and etc.) to the tube. Then add 600 µl GLB. Add 6 µl RNA carrier to the tube. Pulse vortex for 20 s and incubate at 60 °C for 20 min.

   Note: For cell pellets, add 700 µl GLB directly to the pellet or alternatively, for direct lysis of cells grown in a monolayer, add the appropriate amount of GLB to the cell-culture dish. Collect the lysate with a rubber policeman. Transfer the lysate into a microcentrifuge tube. Vortex to mix, and ensure that no cell clumps are visible.

   Optional: This protocol is specialized for CF DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10 µl Prime-RNase A (Cat NO. EB983013), mix by vortexing, and incubate for 7 min at room temperature before going to step 2.
2. Add 600 µl ethanol (100%) to the lysate, mix by pulse vortexing for 20 s, then centrifuge briefly.
3. Gently, pipette half of the mixture to a Micro Column placed in a 2 ml collection tube. Centrifuge at 8000 rpm for 1 min. Discard flow-through and place the Micro Column in the collection tube.

   Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
4. Gently, pipette the remaining mixture to the Micro Column placed in the 2 ml collection tube. Centrifuge at 8000 rpm for 1 min. Discard flow-through and place the Micro Column in the collection tube.

   Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
5. Add 650 µl BWB1 and centrifuge for 1 min at 8000 rpm, discard the flow-through and place the Micro Column in the previous collection tube.
6. Add 750 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the Micro Column in a new clean 1.5 ml 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 flowthrough, place the column back in a collection tube and centrifuge for another 1 min at 14000 rpm.
7. Pipette 30 µl RRB directly onto Micro Column. Incubate at room temperature for

3-5 min. Centrifuge it at 12000 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 DNA yield	Insufficient lysis	• Please refer to Table 2 to apply best match for size of starting material and amount of lysis buffer.
		• Make sure to do pulse-vortexing vigorously after addition of lysis buffer and RJ-Protease.
		• Incubate mixture of the sample and lysis buffer for an additional 15-20 min at 56° C.
	Too few cells in the sample	• Do the test with new samples.
	Sample was not mixed before processing	• Sample should be in suspension. So, make sure to mix before starting the process.
	Incomplete lysing of cells	• 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.
Degradation	Sample contaminated with DNase	• Be sure to do the process in accordance with the reference protocol.

Poor DNA Quality	RNA can be copurified with the genomic DNA	• RNase treatment can be performed.
	Incomplete cell lysis	• Incubate sample with lysis buffer and RJ-Protease for an extra 5-10minutes.
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

Ordering Information

Category	Product name	Cat NO.	Size
DNA Technology	DNJia Micro Kit	DN013070	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; +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: Yield and Purity of DNA

The absorbance of DNA can be measured by any spectrophotometer. The ratio of absorbance at 260 nm and 280nm is used to evaluate the purity of DNA. Pure DNA has an A260/A280 ratio of 1.7–2.0 and also a symmetric peak of absorbance at 260 nm. If the ratio is lower in either case, it may indicate the presence of contamination. Proteins have absorbance at 280 nm. EDTA, carbohydrate and phenol all have absorbance near 230 nm.

Appendix 2: 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: +9821910707

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.

Factory address:
No. 2, Farvardin St., Fernan St., Shahr-e-Qods, 3753146130, Tehran, IRAN.

Tel: 021 91070705