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ROJETECHNOLOGIES

DNJia AmnioPure Kit

DNA isolation based on silica technology

- MiniPrep

For DNA Isolation from

Amniotic Fluid

By ROJE

Edition, 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.

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Kit Content

Component	50 preps	100 preps
GLB	10 ml	20 ml
BWB1 (concentrate)	16 ml	2 X 16 ml
BWB2 (concentrate)	12 ml	2 X 12 ml
RRB	5 ml	10 ml
RJ-Protease	1.25 ml	2 x 1.25 ml
HiPure DR Column	50	100
Collection Tube	50	100

Storage

Shipment condition is checked by ROJETechnologies. After arrival, all reagents should be kept dry, at room temperature. We suggested to store RJ-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 AmnioPure Kit provides the components and procedures necessary for purifying genomic DNA from amniotic fluid. Notice that, DNJia AmnioPure 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

DNJia AmnioPure 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 AmnioPure Kit provides a rapid, careful, convenient and phenol-free method for high quality genomic DNA isolation from amniotic fluid. The procedure is based on spin column technology, which takes less than 20 minutes. DNJia AmnioPure 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 AmnioPure Kit is designed for isolating DNA from 5-6 ml 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 specific 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
- 1.5 ml 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
Processing	Manual
Sample amount	Up to 5-6 ml
Operation time per reaction	Less than 20 Min
Typical yield	Varies
Average purity	A260/A280= 1.7-2.0
Size of DNA purified	≈ 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

Sample	Size of Starting Material	Lysis Buffer Amount
Amniotic fluid	5-6 ml	200 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 3. 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 3: Washing buffer preparation

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

Protocol

Isolation of Genomic DNA (based on silica technology)

Sample type:

- Amniotic fluid

Some tips to know:

- All centrifugation steps are carried out at room temperature (15–25°C).
- If RNase treatment is desired, Prime-RNase can be ordered separately from ROJETechnologies, Cat NO. EB983013.
- 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.

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 200 µl GLB to the pellet then 25 µl RJ-Protease. Pulse vortex until the pellet is dissolved thoroughly incubate at 56 °C for 12 min, pulse vortex for 15 s every 6 min during the incubation.
3. Add 200 µl ethanol (96-100%) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.
4. Gently, pipette the mixture to a HiPure DR 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 HiPure DR Column in to the collection tube.
5. Add 500 µl BWB1 and centrifuge for 1 min at 8000 rpm, discard both the flow-through and the collection tube. Place back the HiPure DR Column in to the collection tube.

6. Add 500 μ l BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR 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.

7. Pipette 25-50 μ l RRB directly onto HiPure DR column. Incubate at room temperature for 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Do the test with new samples.
	Sample was not mixed before processing	<ul style="list-style-type: none"> • Sample should be in suspension. So, make sure to mix before starting the process.
	Incomplete lysing of cells	<ul style="list-style-type: none"> • Repeat the reaction once more and make sure to mix the sample and lysis buffer completely by pulse-vortexing.
	Reagents not applied correctly	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 \geq 7.0.
Degradation	Sample contaminated with DNase	<ul style="list-style-type: none"> • Be sure to do the process in accordance with the reference protocol.
Poor DNA Quality	RNA can be copurified with the genomic DNA	<ul style="list-style-type: none"> • RNase treatment can be performed.
	Incomplete cell lysis	<ul style="list-style-type: none"> • Incubate sample with lysis buffer and RJ-Protease for an extra 5-10 minutes.
DNA does not perform well in downstream applications	DNA was not washed with the provided washing buffer	<ul style="list-style-type: none"> • Ensure the column was washed once with prepared BWB1 and once more with prepared BWB2, respectively.
	Ethanol carryover	<ul style="list-style-type: none"> • Ensure that the traces of ethanol before rehydration step is removed

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 280 nm 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.

Ordering Information

Category	Product name	Cat NO.	Size
DNA Technologies	DNJia AmnioPure Kit	DN983044	50 preps
	DNJia AmnioPure Kit	DN983045	100 preps
Related Products	DNall Plus Kit	DN983048	50 preps
	DNall Plus Kit	DN983049	100 preps
	DNJia Plus Tissue & Bacteria Kit	DN983050	50 preps
	DNJia Plus Tissue & Bacteria Kit	DN983051	100 preps
	DNJia Blood & Cell Kit	DN983025	50 preps
	DNJia Blood & Cell Kit	DN983026	100 preps
	DNJia Plus Blood & Cell Kit	DN983047	50 preps
	DNJia Plus Blood & Cell Kit	DN983046	100 preps
	DNSol, MiniPrep	DN983002	50 preps
	DNSol, MiniPrep	DN983003	100 preps
	DNSol, MiniPrep	DN983004	200 preps
	DNSol, MidiPrep	DN983014	50 preps
	DNSol, MaxiPrep	DN983018	50 preps
	DNSol Clotted Blood Kit	DN983032	50 preps
	DNJia FFPE Tissue Kit	DN983057	50 preps
	DNJia FFPE Tissue Kit	DN983058	100 preps
	Sor	LD983005	2 ml
RJ-Protease, Recombinant (20mg/ml)	EB983121	1 ml	

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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