ROJETECHNOLOGIES

www.rojetechnologies.com

DNSol Clotted Blood Kit

DNA isolation based on solution

- MiniPrep

For DNA Isolation from

Clotted Blood

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 4
Storage 4
Intended Use 4
Guarantee & Warranty 4
Notice to Purchaser 4
Warning and Precautions 5
Quality Control 5
Description 5
Procedure 5
Equipment & Reagents to Be Supplied by User 5
Applications 6
Features 6
Before Start 7
Protocol 7
Troubleshooting 9
Ordering Information 10
Technical Assistance 10
Appendix 1: Yield and Purity of DNA 10
Appendix 2: Convert RPM to RCF (centrifuge) 11
Factory address 11

Kit Content

Component	50 preps
RBC Lysis Buffer, DNSol Edition	100ml
ROS	25ml
RRB	5ml
Prime-RNase A	250µl
RJ-Protease	1ml

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

DNSol Clotted Blood Kit provides the components and procedures necessary for purifying genomic DNA from clotted blood. Notice that, DNSol Clotted Blood 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

DNSol Clotted Blood 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

DNA isolation from clotted blood can be difficult and often results in low yields. However, ROJETechnologies provides DNSol Clotted Blood Kit, a timesaving, reliable and meticulous method for high quality genomic DNA isolation from Clotted blood. DNSol Clotted Blood Kit is manual and based on Solution for isolation of concentrated, highly purified, intact genomic DNA, which is suitable to be used for a variety of downstream applications such as PCR analysis and restriction endonuclease digestions. The solution-based system minimizes DNA fragmentation that may be problematic in spin-column / filtration-based methods.

Procedure

In DNSol Clotted Blood Kit, WBCs are separated after lysing red blood cells by RBC Lysis buffer, then WBCs are incubated with RJ-Protease and ROS Buffer. Aqueous phase is separated using phenol-chloroform. Aqueous phase is used in order to obtain a pure nucleic acid. DNA will be precipitated using isopropanol and washed away by using %70 ethanol to eliminate salt contaminations. Finally, DNA is rehydrated using RRB. Purified DNA has A260/A280 ratios approximately 1.7-2 and A260/A230 ratios of 1.8-2.2. The rehydrated DNA is stable for several months at 4 °C and greater than 1 year at -20 °C.

Equipment & Reagents to Be Supplied by User

- Molecular biology grade ethanol (%96-100)
- Phenol

- Chloroform
- Isopropanol
- 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
- Cloning

Features

Specific features of DNSol Clotted Blood Kit are listed here in Table 1.

Table 1. DNSol Clotted Blood Kit features and specifications

Features	Specifications
Elution volume	50µl
Technology	Solution based
Format	Salting out
Main sample type	Clotted Blood
Processing	Manual
Sample amount	400µl clotted blood
Minimum blood input	200µl
Maximum blood input	400µl
Operation time per reaction	Less than 2 hours
Typical yield	Varies

Average purity	A260/A280= 1.7-2.0
Size of DNA purified	≈ 50 Kb

Before Start

- If ROS or RBC Lysis Buffer or PPS forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and won't influence the efficiency of buffer.

Maximize DNA Yield

To obtain higher yield of DNA, it is important to follow protocol carefully and pay attention to sample size and suitable lysis buffer recommended for samples. Notice that for all samples, the white blood cells must be completely homogenized during cell lysis for maximum yields.

Protocol

Isolation of Genomic DNA (based on solution)

Sample type: Clotted Blood

Some tips to know:

- All centrifugation steps are carried out at room temperature (15–25°C).
- If any buffer (ROS or RBC Lysis Buffer) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

- Add 1ml RBC lysis buffer to 400µl clotted blood in microtube, invert 5 times, vortex 10 s at high speed and centrifuge at 13000 rpm for 3min.
- Discard supernatant and add 1 ml RBC lysis buffer to the pellet, invert 5 times, vortex for 10 s at high speed and centrifuge at 10000 rpm for 2min.
- Discard supernatant. Add 500µl ROS and then 20µl RJ-Protease. Mix thoroughly by pulse vortexing for 30s, then incubate at 56°C for 30-60min until the sample is completely lysed. Pulse vortex every 10min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.
- Add 200µl phenol %50 and then 200µl chloroform. Vigorously shake it for 30 s. Then pulse vortex for 15 s and centrifuge at 13000 rpm for 10min.

- Transfer the supernatant to a new tube. Add 5µl Prime-RNase A to the isolated aqueous phase. Then pulse vortex for 5 s and incubate for 10min at room temperature, 25 °C.
- Add equal volume of isopropanol to the microcentrifuge tube. Invert 10-15 times rapidly. Centrifuge at 12000 rpm for 1min.

Note: The DNA should be visible as a small white pellet.

- Discard supernatant, aspirate the pellet. Add 600µl ethanol %70 to the pellet; centrifuge at 10000 rpm for 2min.
- Discard supernatant and aspirate the pellet. Then, add 50µl RRB. Mix by pipetting until the pellet is dissolved completely. Alternatively, you can vortex for 10 s after adding the RRB, then incubate at 37°C for 10min (or 20min at room temperature, 25 °C); afterward vortex for 10s, to dissolve the DNA. The DNA is ready for further applications; you can use 2-5µl of it for PCR reaction.

Note: Do not dry the pellet and add RRB immediately.

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	• Not forget to add appropriate lysis buffer in accordance with the reference protocol.
		• Make sure to do pulse-vortexing vigorously after addition of lysis buffer.
	Too few white blood cells in the sample	• Do the test with new samples.
	Incomplete lysing of WBC's	• 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.
		• 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.
	Too old sample	• Old samples stored at inappropriate conditions always yield sheared DNA.
DNA does not perform well in downstream applications	Ethanol carryover	• Ensure that the traces of ethanol before rehydration step is removed.

Ordering Information

Category	Product name	Cat NO.	Size
DNA Technologies	DNSol Clotted Blood Kit	DN983032	50 preps
	DNSol, MiniPrep	DN983003	100 preps
	DNSol, MidiPrep	DN983014	50 preps
	DNall Plus Kit	DN983066	25 preps
	DNall Plus Kit	DN983049	100 preps
	DNJia AmnioPure Kit	DN983045	100 preps
	DNJia Plus Blood & Cell Kit	DN983047	100 preps
	DNJia FFPE Tissue Kit	DN983057	50 preps
	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: Yield and Purity of DNA

The absorbance of DNA can be measured by any spectrophotometer. The ratio of absorbance at 260nm and 280nm is used to evaluate the purity of DNA. Pure DNA has an A260/A280 ratio of 1.8–2.0 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: +982191070705

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