ROJETECHNOLOGIES

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DNSol Kit

DNA isolation based on solution

- MiniPrep
- MidiPrep
- MaxiPrep

For DNA Isolation from

Blood
Buffy coat

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.

Contents

Kit Content

DNSol (MiniPrep)

Component	50 preps	100 preps	200 preps
RBC Lysis Buffer	2 x 50 ml	4 x 50 ml	8 x 50 ml
ROS	20 ml	40 ml	2 x 40 ml
PPS	7 ml	14 ml	28 ml
RRB	5 ml	10 ml	20 ml
Isopropanol	30 ml	60 ml	2 x 60 ml

DNSol (MidiPrep)

Component	50 preps
RBC Lysis Buffer	8 x 50 ml
ROS	2 x 32 ml
PPS	28 ml
RRB	10 ml

DNSol (MaxiPrep)

Component	50 preps
RBC Lysis Buffer	1200 ml
ROS	3 x 42 ml
PPS	54 ml
RRB	25 ml

Storage

Shipment condition is checked by ROJETechnologies. After arrival, all reagents should be kept dry at room temperature. When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

DNSol Kit provides the components and procedures necessary for purifying genomic DNA from blood and buffy coat. Notice that, DNSol 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 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

Obtaining high-quality, intact DNA is often the first and most critical step in many fundamental molecular biology applications. Purification is based on thoroughly modified salting out method, designed to reach highly efficient and pure genomic DNA. DNA can be purified in less than 25 minutes. ROJETechnologies kits maximize process efficiency and downstream performance.

Procedure

In DNSol Kit, for DNA isolation from whole blood, WBCs are separated after lysing RBCs, and then they are lysed using special ionic detergents. Contaminants such as protein are precipitated using an efficient salting out procedure; DNA will be precipitated using absolute ethanol or isopropanol. DNA is washed using 70% ethanol to wash away the salts and then it is rehydrated using freshly made rehydration buffer. Purified DNA has A260/A280 ratios approximately 1.8 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

- Ethanol (96-100%)
- Pipets and pipet tips
- 1.5 ml Microtube
- Vortex
- Centrifuge
- Micro centrifuge
- Dry Heat Block/ Water Bath
- Proteinase K / RJ-Protease for DNA isolation from buffy coat

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 Kit are listed here in Table 1.

Table 1. DNSol Kit features and specifications

Features	Specifications
Elution volume	50-200µl
Technology	Solution based
Format	Salting out
Main sample type	Fresh whole blood, buffy coat
Processing	Manual
Sample amount (for MiniPrep)	Up To 300 µl blood or buffy coat
Minimum blood input (for MiniPrep)	100 µl
Maximum blood input (for MiniPrep)	300 µl
Operation time per reaction	25 Min
Typical yield	Varies
Average purity	A260/A280= 1.7-2.0
Size of DNA purified	≈ 50 Kb

Sample Preparation

Blood

- Its recommended to collect blood in standard collection tube with EDTA as anticoagulants. Other anticoagulants such as ACD (citrate) and heparin may be used. Notice, heparin has been shown to inhibit Taq polymerase activity and its recommended to be avoided, when possible.
- For optimum results, do not store sample at 4 °C for more than 5 days.
- Samples stored at -75 °C could be used for at least 2 years. Before use, thaw quickly in a 37 °C water bath and keep sample on ice until use.

Buffy Coat

Buffy coat is a leukocyte-enriched fragment of whole blood. Buffy coat contains the majority of the white blood cells and platelets as well as an equivalent amount of genomic DNA (gDNA) when compared to whole blood. For collecting buffy coat, it is recommended to do as follows:

- Use fresh blood that was collected in standard collection tubes with EDTA as anticoagulants.
- Centrifuge 2.5 ml of whole blood for 10 minutes at 2,500 g
- Remove upper plasma portion and carefully collect the cells at the interface by using a pipette and place in a separate tube (An approximately tenfold concentration of cells is obtained using this technique 200 µl buffy coat from 2.5 ml blood).

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. It is good to know that:

- Yield and quality of the purified DNA depend on blood storage conditions. For best results, blood should be stored at room temperature for no longer than 24 hours or at 4°C for no longer than 5 days. For long-term storage, freeze blood at –70 °C. Storing blood at -20 °C, can compromise the integrity of the sample, then results in reducing yields and quality of DNA.

Protocols

Protocol 1: Isolation of Genomic DNA (based on solution)

Type: MiniPrep

Some tips to know:

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

Process

1. Add 1 ml RBC lysis buffer to 300 µl blood in microtube, invert 5 times, vortex 10 s at high speed and centrifuge at 13000 rpm for 3 min.
2. 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 2 min.

   Note: You will see a small pellet at the bottom of the microtube.
3. Discard supernatant. Aspirate the pellet in a way that about 20 µl of supernatant remains in the microtube, vortex for 10 s to resuspend the pellet, add 400 µl ROS to pellet and vortex for 20 s at high speed until the pellet is dissolved thoroughly.
4. Add 100 µl PPS; shake vigorously for 5 s, vortex for 10-15 s at high speed then shake vigorously again for few s and centrifuge at 13000 rpm for 5 min.

   Note: The supernatant should be completely clear. If not add more 35 µl of PPS to the microtube, shake vigorously and vortex the microtube at high speed then centrifuge at 13000 rpm for 2 min.
5. Pour supernatant to clean microtube; add 600 µl isopropanol to supernatant. Invert 10 - 20 times rapidly. Centrifuge at 12000 rpm for 1 min.

   Note: Be sure the protein pellet is not dislodged during pouring.
6. Discard supernatant, aspirate the pellet. Add 600 µl ethanol 70% to the pellet; centrifuge at 10000 rpm for 2 min.
7. Discard supernatant, aspirate the pellet, and add 50 µl 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 10 min (or 20 min at room temperature, 25 °C); afterward vortex for 10 s, to dissolve the DNA.
8. The DNA is ready for further applications, you can use 2-5 µl of it for PCR reaction.

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

Protocol 2: Isolation of Genomic DNA (based on solution)

Type: MidiPrep

Some tips to know:

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

Process

1. Add 6 ml RBC lysis buffer to 2 ml blood in tube, invert 5 times, vortex 10 s at high speed and centrifuge at 4000 rpm (2000 g) for 3 min.
2. Discard supernatant and add 2 ml RBC lysis buffer to the pellet, 5 times, vortex 10 s at high speed and centrifuge at 4000 rpm (2000 g) for 2 min.

   Note: You will see a small pellet at the bottom of the tube.

3. Discard supernatant. Vortex for 10 s to resuspend the pellet, add 1250 µl ROS to pellet and vortex for 20 s at high speed until the pellet is dissolved thoroughly.
4. Add 500 µl PPS; shake vigorously 5 s, vortex for 15-20 s at high speed then shake vigorously again for few seconds and centrifuge at 4000 rpm (2000 g) for 7 min.

   Note: The supernatant should be clear. If not add more 50 µl of PPS to the tube, shake vigorously and vortex tube at high speed then centrifuge at 4000 rpm (2000 g) for 2 min.

5. Pour supernatant to clean tube, add 3 ml absolute ethanol to supernatant. Invert 5 times slowly, put the tube in freezer or refrigerator for 2 min.

   Note: Be sure the protein pellet is not dislodged during pouring.

6. Separate the DNA by micropipette 1000, which is set on 100 µl and transfer the DNA to a clean microtube. Add 1000 µl ethanol 70% to the pellet; centrifuge at 11000 rpm for 2 min.
7. Discard supernatant, aspirate the pellet and add 200 µl RRB. Mix by pipetting until the pellet is dissolved completely, continue pipetting the DNA for 1 min. **Alternatively**, you can vortex for 10 s after adding the RRB, then incubate at 37 °C for 10 min (or 20 min at room temperature, 25 °C); afterward vortex for 10 s.
8. The DNA is ready for further applications, you can use 2-5 µl of it for PCR reaction.

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

Protocol 3: Isolation of Genomic DNA (based on solution)

Type: MaxiPrep

Some tips to know:

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

Process

1. Add 20 ml RBC lysis buffer to 5 ml blood in tube, invert 5 times, vortex 10 s at high speed and centrifuge at 3200 g (4500 rpm) for 5 min.
2. Discard supernatant and add 4 ml RBC lysis buffer to the pellet, invert 5 times, vortex 10 s at high speed and centrifuge at 3200 g (4500 rpm) for 3 min.

   Note: You will see a small pellet at the bottom of the tube.

3. Discard supernatant. Vortex for 20 s to resuspend the pellet, add 2500 µl ROS to pellet and vortex for 20-60 s at high speed until the pellet is dissolved thoroughly, then incubate at room temperature for 5 min.
4. Add 1ml PPS; shake vigorously 10 s, vortex for 15-20 s at high speed then shake vigorously again for few s and centrifuge at 3200 g (4500 rpm) for 12 min.

   Note: The supernatant should be clear. If not add more 50 µl of PPS to the tube, shake vigorously and vortex tube at high speed then centrifuge at 3200 g (4500 rpm) for 2 min.

5. Pour supernatant to clean tube, add 5 ml absolute ethanol to supernatant. Invert 5 times slowly, put the tube in freezer or refrigerator for 2 min.

   Note: Be sure the protein pellet is not dislodged during pouring.

6. Separate the DNA by micropipette 1000, which is set on 100 µl and transfer the DNA to a clean microtube. Add 1000 µl ethanol 70% to the pellet; centrifuge at 11000 rpm for 2 min.
7. Discard supernatant, aspirate the pellet and add 500 µl to 1ml RRB, based on your desired concentration. Mix by pipetting until the pellet is dissolved completely, continue pipetting the DNA for 1 min. **Alternatively**, you can vortex for 10 s after adding the RRB, then incubate at 37 °C for 10 min, afterward vortex for 10 seconds
8. The DNA is ready for further applications, you can use 25 µl of it for PCR reaction.

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

Protocol 4: Isolation of Genomic DNA (based on solution)

Sample type: Buffy coat

Some tips to know:

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

Process

1. Add 1 ml RBC lysis buffer to 300 µl buffy coat in microtube, invert 5 times, vortex 10 s at high speed and centrifuge at 13000 rpm for 3 min.
2. 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 2 min.

   Note: You will see a small pellet at the bottom of the microtube.
3. Discard supernatant. Aspirate the pellet in a way that about 20 µl of supernatant remains in the microtube, vortex for 10 s to resuspend the pellet.
4. Add 400 µl ROS to pellet and then 20 µl proteinase K or RJ-Protease (order by Cat No. EB983018, EB983121). Mix thoroughly by pulse vortexing for 30 s, then incubate at 56°C for 15-30 min until the pellet is completely lysed. Pulse vortex every 5 min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.
5. Add 200 µl PPS; shake vigorously for 10 s, vortex for 10-15 s at high speed then shake vigorously again for few s and centrifuge at 13000 rpm for 5 min.

   Note: The supernatant should be completely clear. If not add more 35 µl of PPS to the microtube, shake vigorously and vortex the microtube at high speed then centrifuge at 13000 rpm for 2 min.
6. Pour supernatant to clean microtube; add 600 µl isopropanol to supernatant. Invert 10 - 20 times rapidly. Centrifuge at 12000 rpm for 1 min.

   Note: Be sure the protein pellet is not dislodged during pouring.
7. Discard supernatant, aspirate the pellet. Add 600 µl ethanol 70% to the pellet; centrifuge at 10000 rpm for 2 min.
8. Discard supernatant, aspirate the pellet, and add 100-200 µl 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 10 min (or 20 min at room temperature, 25 °C); afterward vortex for 10 s, to dissolve the DNA.

   Note: Do not dry the pellet and add the solvent 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	The blood sample was too old	• Please refer to sample preparation guidelines.
	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 blood samples.
	Whole blood sample was not mixed before processing	• White blood cells should be in suspension. So, make sure to mix whole blood samples before processing.
	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.
	Inappropriate sample collection or storage of starting material	• Please refer to sample preparation guidelines.
	Too old sample	• Old samples stored at inappropriate conditions always yield sheared DNA.
Poor DNA Quality	RNA can be copurified with the genomic DNA	• RNase treatment can be performed.
DNA does not perform well in downstream applications	Ethanol carryover	• 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 1.8–2.0 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.

Ordering Information

Category	Product name	Cat NO.	Size
DNA Technologies	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
Related Products	DNJia Blood & Cell Kit	DN983025	50 preps
	DNJia Blood & Cell Kit	DN983026	100 preps
	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 AmnioPure Kit	DN983044	50 preps
	DNJia AmnioPure Kit	DN983045	100 preps
	DNJia Plus Blood & Cell Kit	DN983047	50 preps
	DNJia Plus Blood & Cell Kit	DN983046	100 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.

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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