Quick Protocol

DNSol Kit

DNA isolation based on solution

- MiniPrep
- MidiPrep
- MaxiPrep

For DNA Isolation from

Blood
Buffy coat

Kit Content

DNSol (MiniPrep)

Component	100 preps
RBC Lysis Buffer	4 x 50ml
ROS	40ml
PPS	14ml
RRB	10ml
Isopropanol	60ml

DNSol (MidiPrep)

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

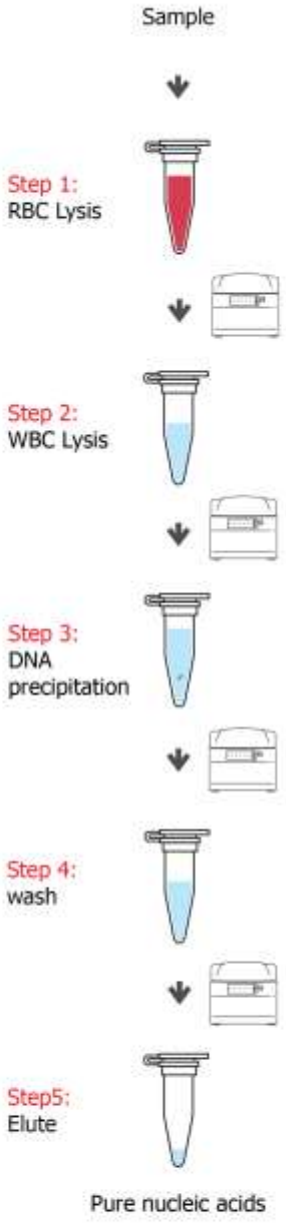
DNSol (MaxiPrep)

Component	50 preps
RBC Lysis Buffer	2×500ml
	200ml
ROS	3 x 42ml
PPS	54ml
RRB	25ml

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.

Procedure of solution-based DNA isolation in quick look


Sample
Step 1:
RBC Lysis
Step 2:
WBC Lysis
Step 3:
DNA
precipitation
Step 4:
wash
Step5:
Elute
Pure nucleic acids

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

- 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.
- 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.
- 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.
- 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.
- 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.
- Discard supernatant, aspirate the pellet. Add 600 µl ethanol 70% to the pellet; centrifuge at 10000 rpm for 2 min.
- 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.
- 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

- 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.
- 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.

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

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

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

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

- Separate the DNA by micropipette 1000, which is set on 100µl and transfer the DNA to a clean microtube. Add 1000µl ethanol %70to the pellet; centrifuge at 11000 rpm for 2min.
- Discard supernatant, aspirate the pellet and add 200µl RRB. Mix by pipetting until the pellet is dissolved completely, continue pipetting the DNA for 1min. **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.
- 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 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

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

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

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

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

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

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

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

- The DNA is ready for further applications, you can use 25µl 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

- Add 1ml RBC lysis buffer to 300µl buffy coat in microtube, invert 5 times, vortex 10s at high speed and centrifuge at 13000 rpm for 3min.
- Discard supernatant and Add 1ml RBC lysis buffer to the pellet, invert 5 times, vortex for 10s at high speed and centrifuge at 10000 rpm for 2min.

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

  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 2min.
- Pour supernatant to clean microtube; add 600µl isopropanol to supernatant. Invert 10 - 20 times rapidly. Centrifuge at 12000 rpm for 1min.

  Note: Be sure the protein pellet is not dislodged during pouring.
- Discard supernatant, aspirate the pellet. Add 600µl ethanol %70 to the pellet; centrifuge at 10000 rpm for 2min.
- Discard supernatant, aspirate the pellet, and add 100-200µl RRB. Mix by pipetting until the pellet is dissolved completely. **Alternatively,** you can vortex for 10s 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.

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