Performance Validation Report

SolPure Blood DNA Kit

Large-Volume Blood DNA Extraction by Salting-Out Method

Report Summary: This report evaluates SolPure Blood DNA Kit for purification of high-molecular-weight genomic DNA from large-volume and challenging blood samples. DNA purity, yield, integrity and PCR compatibility were assessed using OD measurement, agarose gel electrophoresis and PCR amplification.

1. Introduction

Blood is one of the most widely used human sample materials. Blood samples contain abundant genomic DNA and are an important source material for molecular biology, disease research and testing workflows. Extraction of genomic DNA from blood is therefore a routine task for many laboratories.

Common methods for blood genomic DNA extraction include phenol/chloroform extraction, silica column purification, silica magnetic particle purification, ion-exchange column purification and salting-out extraction. Each method has its advantages and limitations. Phenol/chloroform extraction can be prepared in a laboratory at low cost, but it involves hazardous organic chemicals and may produce DNA with insufficient purity. Commercial silica column, magnetic bead and ion-exchange kits provide stable yield and purity, but they may become costly when processing large-volume blood samples, especially 1-10 mL inputs.

SolPure Blood DNA Kit is based on an improved salting-out method and is designed as an economical and scalable option for extracting genomic DNA from large-volume blood samples. In this validation, anticoagulated blood samples were processed using SolPure Blood DNA Kit, and the purified DNA was evaluated by gel electrophoresis, OD measurement and PCR to assess purity and integrity.

2. Experimental Design

Different blood sample types were selected for DNA extraction. Each sample type was processed in duplicate.

- 1 mL human anticoagulated blood
- 10 mL human anticoagulated blood
- 3 mL long-term stored anticoagulated blood (-20°C storage for three years)
- 5 mL porcine anticoagulated blood
- 1 mL coagulated porcine blood

Pretreatment of Coagulated Porcine Blood

Two grams of coagulated porcine blood were transferred into a 50 mL centrifuge tube and homogenized twice for 30 seconds using a mechanical homogenizer to re-disperse the coagulated sample. A glass homogenizer may also be used. One milliliter of the homogenized blood was then processed according to the normal blood protocol.

3. Extraction Procedure

Step	Operation
1	Transfer the required volume of blood sample into a 15 mL or 50 mL centrifuge tube.
2	Add 3 volumes of RBC Lysis Buffer and mix by vortexing.
3	Incubate at room temperature for 5 minutes, inverting once during incubation.
4	Centrifuge at 2,000 x g for 5 minutes. Carefully discard the supernatant. For 3 mL long-term stored blood, remove the supernatant by aspiration and retain approximately 1 mL residual liquid with the pellet.
5	Vortex to resuspend the white blood cell pellet.
6	Add Cell Lysis Buffer equal to the original blood volume and mix by vortexing.
7	Incubate at 37°C for 10-120 minutes. For long-term stored blood, incubate for 120 minutes.
8	Add RNase and incubate at 37°C for 30 minutes.

9	Add 1/3 volume of Protein Precipitate Solution and vortex vigorously for 30 seconds.
10	Centrifuge at 2,000 x g for 5 minutes at room temperature.
11	Transfer the supernatant and add an equal volume of isopropanol. For long-term stored blood, add 40 µL glycogen. Mix by inverting 30-50 times.
12	Centrifuge at 2,000 x g for 5 minutes at room temperature.
13	Carefully discard the supernatant, add 70% ethanol, and invert several times to wash the DNA pellet.
14	Centrifuge at 2,000 x g for 5 minutes at room temperature.
15	Carefully discard the supernatant and invert the tube on absorbent paper for 5-10 minutes to dry the pellet.
16	Add Elution Buffer, vortex for 10 seconds, and incubate at 65°C for 1 hour.
17	Transfer to 4°C and leave overnight to allow complete DNA dissolution.

4. Results

4.1 DNA Purity and Yield

Purified DNA was diluted 11-fold with Buffer TE and measured using a Beckman DU640 spectrophotometer at OD260, OD280, OD230 and OD320. The results showed that the purified DNA reached a purity ratio of 1.8 or above. Dissolution volumes were not listed in the original data table.

Sample	OD260	OD280	OD230	Purity	Yield (µg)
1 mL human blood	0.1412	0.0796	0.1049	1.8	38.8
1 mL human blood	0.1415	0.0804	0.0881	1.8	38.9
10 mL human blood	0.1511	0.0851	0.1289	1.8	332.4
10 mL human blood	0.1763	0.0894	0.1504	2.0	387.9
3 mL stored blood	0.1889	0.0989	0.1412	1.9	155.8
3 mL stored blood	0.1921	0.0952	0.1443	2.0	158.5
5 mL porcine blood	0.2894	0.1529	0.1281	1.9	397.9
5 mL porcine blood	0.2999	0.1703	0.1412	1.8	412.4
1 mL coagulated blood	0.2332	0.1312	0.1741	1.8	64.1
1 mL coagulated blood	0.2294	0.1219	0.1702	1.9	63.1

4.2 DNA Integrity

Two microliters of purified DNA were loaded on a 0.8% agarose gel and electrophoresed at 80 V for 30 minutes. Lambda DNA/Hind III Marker was used as the reference marker. The gel results showed high integrity genomic DNA without visible smearing, with DNA fragments larger than 23 kb.

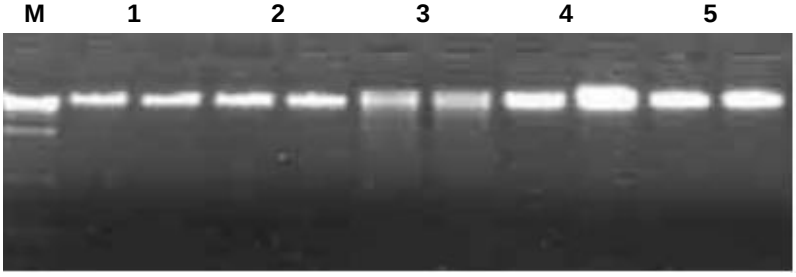

Figure 1. DNA integrity analysis by agarose gel electrophoresis. M: Lambda DNA/Hind III Marker; Lane 1: 1 mL human anticoagulated blood; Lane 2: 10 mL human anticoagulated blood; Lane 3: 3 mL long-term stored anticoagulated blood (-20°C for three years); Lane 4: 5 mL porcine anticoagulated blood; Lane 5: 1 mL coagulated porcine blood.

4.3 PCR Validation

Human PP1 primers, generating an approximately 600 bp amplicon, and porcine-specific primers, generating an approximately 300 bp amplicon, were used to evaluate PCR compatibility of the purified DNA. The electrophoresis results showed successful amplification from the purified DNA samples.

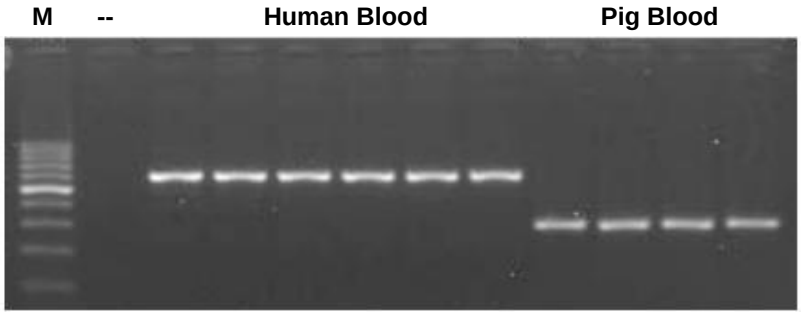

Figure 2. PCR validation of purified DNA. "-" indicates negative control. M: 100 bp Marker. Human blood samples were amplified using human PP1 primers; porcine blood samples were amplified using porcine-specific primers.

5. Conclusion

The validation results demonstrate that SolPure Blood DNA Kit can purify high-molecular-weight genomic DNA from different blood sample types, including large-volume human blood, long-term stored blood, porcine blood and pre-homogenized coagulated blood. The purified DNA showed good purity, high yield, clear high-molecular-weight bands and compatibility with PCR amplification. These results support the use of the kit for large-volume blood genomic DNA extraction, archiving and downstream molecular analysis workflows.

6. Technical Notes and Frequently Asked Questions

1. What are the key factors affecting DNA purity?

The salting-out procedure is simple, but it is sensitive to the ratio between blood volume and reagent volume. Sufficient amounts of Cell Lysis Buffer and Protein Precipitate Solution are critical. After adding Protein Precipitate Solution, vigorous vortexing for 30 seconds is required to fully precipitate proteins. Insufficient vortexing can clearly affect DNA purity.

2. How long should RNase A digestion be performed, and how does RNA contamination occur?

Isopropanol precipitation does not selectively recover DNA over RNA. Therefore, RNase digestion is important. RNase treatment for approximately 15-40 minutes is usually sufficient. Only after RNA is fully digested into very small fragments will it remain unprecipitated during isopropanol precipitation, reducing RNA contamination in the final DNA.

3. What size DNA fragments can be obtained with this kit?

This method can obtain high-molecular-weight genomic DNA. Pulse-field electrophoresis data indicate that DNA obtained by this method is approximately 50-150 kb.

4. How long does DNA dissolution require?

The DNA obtained by this method has a large molecular size and may be difficult to dissolve. It is recommended to add Elution Buffer or Buffer TE, incubate at 65°C for 1 hour, and then transfer to 4°C overnight to ensure complete dissolution. When DNA yield is low (<0.1 μg/μL), 30 minutes at 65°C is generally sufficient.

5. Why do some samples form visible fibrous precipitates after isopropanol addition while others do not?

This is mainly related to blood storage condition. Normally stored blood usually produces visible fibrous DNA precipitates. Blood stored for too long, such as more than 1 month at -20°C or more than 1 week at 4°C, may have degraded DNA and generally does not form visible fibrous precipitates.

6. How can yield and stability be improved for old or improperly stored blood samples?

Old blood samples may have hemolysis or DNA degradation caused by poor storage conditions, resulting in 2- to 10-fold lower DNA yield than normal blood. Because isopropanol precipitation is inefficient for recovering small amounts of DNA, addition of glycogen (20 mg/mL) can clearly improve yield and stability when processing old or poorly stored blood.

7. Can this kit process bird and fish blood samples?

Yes. Bird and fish red blood cells are nucleated, so the red blood cell lysis step should be greatly reduced or omitted. Experimental data showed that 10 µL chicken blood can produce approximately 20-30 µg DNA.

8. Can this kit extract DNA from coagulated blood samples?

Yes. For coagulated blood samples, the blood must first be fully dispersed using a homogenizer and then processed according to the standard workflow.

9. What are the recommended storage conditions for blood samples?

Blood is generally recommended to be stored at 4°C for no more than 1 week. For longer storage, samples may be kept at -70°C, but genomic DNA extraction quality decreases as storage time increases.

10. Should salting-out or silica-column purification be used for infectious samples?

For large batches of blood samples, the salting-out method can reduce cost substantially. However, salting-out requires white blood cell separation, and this separation step does not inactivate infectious viruses or microorganisms such as hepatitis virus or Staphylococcus aureus. Red blood cell lysis waste and used tubes may therefore carry infectious risk. Silica column methods, such as HiPure Blood DNA Mini Kit, are more expensive but do not require white blood cell separation. Blood can be mixed directly with lysis buffer and protease to rapidly inactivate viruses and other infectious microorganisms, reducing infectious waste and improving operator and laboratory safety. Customers should choose the method according to sample source, infectious risk and budget.

For additional product information, please refer to the SolPure Blood DNA Kit manual.