BloodZol

Cat. No. EE131

Storage: Proteinase K solution at -20°C for one year; others at room temperature (15-25°C) for one year

Description

BloodZol provides an easy and fast method to isolate high quality genomic DNA from 0.1-20 ml of fresh or frozen blood. Isolated DNA is free of contaminants and enzyme inhibitors. Red Cell Lysis Buffer is provided to remove non-nucleated red cells and reduce hemoglobin contamination. Genomic DNA is precipitated with isopropanol.

- High quality, free of contaminants and inhibitors.
- Suitable for EDTA, sodium citrate and heparin-anticoagulated fresh and frozen blood.
- No organic solvents.
- Isolated DNA is suitable for PCR, restriction enzyme digestion and Southern blot.

Kit Contents

Component	EE131-01 (process 50 ml blood)	EE131-02 (process 200 ml blood)
Red Cell Lysis Buffer (RCL)	125 ml	2×250 ml
Lysis Buffer3 (LB3)	30 ml	120 ml
Elution Buffer (EB)	25 ml	80 ml
Proteinase K (20 mg/ml)	250 µl	1 ml

Procedures

All centrifugation steps are carried out at room temperature

(400 µl of blood sample used as example)

1. Add 2.5 times volume of RCL (e.g. use 1 ml of RCL for 400 µl of blood) to blood sample. Mix by inverting several times, and then incubate for 5 minutes at room temperature.
2. Centrifuge the mixture at 10,000×g for 3 minutes. Carefully remove the supernatant, leaving the white cell pellets.
3. Add 200 µl of LB3 and 2 µl of Proteinase K to the pellets. Mix thoroughly immediately by vortexing. Incubate at 65°C for 10 minutes,vortex for several times during this period until the solution becomes clear (if solution does not become completely clear, it means the cell lysis is not complete, which may result in low yield and low purity of DNA. In that case, increase the lysis time until the solution is completely clear).
4. Add 200 µl of isopropanol to the solution. Mix thoroughly by inverting, filamentous or clustered genomic DNA may form at this moment.
5. Centrifuge at 10,000×g for 5 minutes. Carefully remove the supernatant.
6. Add 200 µl of 70% ethanol, vortexing for 5 seconds. Centrifuge at 10,000×g for 5 minutes and carefully remove the supernatant.
7. Repeat step 6 once.
8. Air-dry the DNA pellet until all the liquid completely evaporate. Dissolve DNA by adding 200 µl of EB to it and vortexing at low speed for 5 seconds and incubate for 10 minutes to 1 hour at 65°C, gently tap for several times to facilitate complete solubilization.

Appendix

	Blood volume (μl)						
	100	400	1,000	3,000	5,000	10,000	20,000
Red Cell Lysis Buffer (RCL)	250	1,000	2,500	7,500	12,500	25,000	50,000
Lysis Buffer 3(LB3)	50	200	500	1,500	2,500	5,000	10,000
Proteinase K	0.5	2	5	15	25	50	100
100% isopropanol	50	200	500	1,500	2,500	5,000	10,000
70% ethanol	50	200	500	1,500	2,500	5,000	10,000
Elution Buffer (EB)	50	200	200	300	500	1,000	1,000
Centrifugation speed	10,000×g	10,000×g	3,000×g	3,000×g	3,000×g	3,000×g	3,000×g
Centrifugation time	3/5/5 min	3/5/5 min	10/10/10 min	10/10/10 min	10/10/10 min	10/10/10 min	10/10/10 min

Notes

- All centrifugation steps are carried out at room temperature.
- Repeated freezing and thawing of blood sample can result in shortened DNA fragment and decreased extraction quantity. Additionally, avoid repeated freezing and thawing of purified genomic DNA to minimize DNA shearing and fragmentation.
- Storage of blood sample

Short-term storage: blood samples treated with the anticoagulant can be stored up to 10 days at 2-8°C.

Long-term storage: store anticoagulant-treated blood samples at -70°C (EDTA is recommended to be used as anticoagulant for extracting high molecular weight DNA)

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