BLOOD CLOT DNA EXTRACTION

Materials needed prior to start

- Add 96 - 100% Ethanol to Wash Buffers as indicated on the bottle.
- Set the water bath/heat block at 56°C.
- Reconstitute Proteinase K with Proteinase K Buffer and store at 2 - 8°C.

PROTOCOL

Blood Lysate Preparation

1. Weigh approximately 100 mg of clot and mince the blood clot using a sterile surgical blade.
2. Transfer the clot to a sterile microcentrifuge tube.
3. To the clot, add **500 µl of Blood Lysis Buffer 1** and mix thoroughly by pipetting.
4. Centrifuge at **8000 rpm for 2 mins** at room temperature.
5. Discard the supernatant and add **500 µl of Blood Lysis Buffer 1** again**.**
6. Resuspend the pellet by pipette mixing.
7. Centrifuge at **8000 rpm for 2 mins** at room temperature and discard the supernatant.
8. Add **500 µl of Blood Lysis buffer 2** to the pellet and resuspend by pipette mixing.
9. Add **20 µl of Proteinase K** and resuspend by pipetting.
10. Incubate at **56°C for 30 mins**.
11. Transfer the lysate to a fresh microcentrifuge tube.

DNA Binding and Washing

Note: Vortex the Blood MagNa Mix to ensure complete dispersion of the particles.

12. Add **350 µl of Blood MagNa Mix** and invert the tube 10 - 12 times to mix properly.
13. Incubate the samples at room temperature for 5 mins.
14. Place the tube on MagNa Stand until the solution gets cleared. Discard the supernatant.
15. To the pellet, add **500 µl of Blood Wash Buffer 1**, remove the tube from MagNa Stand and resuspend by pipette mixing *(ensure complete dispersion of the particles)*.

Note: Use 200 µl pipette for better resuspension of the pellet.

16. Place the tube back on MagNa Stand until the solution gets cleared (*30 sec - 1 min*).
17. Discard the supernatant without removing the tube from MagNa Stand (*Make sure that the pellet is not disturbed*).
18. Add **500 µl of Blood Wash Buffer 2** & gently invert mix the tube without removing from MagNa Stand for 5 - 6 times (*surface wash only*).
19. Discard the supernatant without removing the tube from MagNa Stand.
20. Repeat steps 17 - 19.
21. Air dry the pellet without removing the tube from MagNa Stand for 10 - 15 mins.

Note: Do not over dry the pellet.

DNA Elution

22. To the pellet add **50 µl Elution Buffer**, remove the tube from MagNa Stand, and disperse the pellet thoroughly by pipette mixing.
23. Incubate at **56°C for 5 mins.**
24. Place the tube back on MagNa Stand until the solution gets cleared.
25. Carefully transfer the supernatant to a new microcentrifuge tube and discard the MagNa particles.

Downstream applications

DNA obtained from this method is ready to use for any downstream application without any further precipitation or purification step.

Troubleshooting Guide

Observation	Possible Causes	Suggested Solution
Low DNA yield or Poor Quality	Incomplete Lysis	Ensure that the blood clot is minced properly and it is not too old/dried. Check if the sample is stored properly.
		Use suggested amount of Proteinase K for the specified time.
		Ensure proper re-suspension of pellet while adding lysis buffers.
		Make sure that the Lysis incubation temperature and time is followed correctly.
		In some cases when DNA content is very high, the lysate may appear slimy. Ensure thorough pipette mixing during lysis and before adding MagNa Mix in such circumstances.
	Incorrect reagent volumes were used	Use the exact volumes of reagents mentioned in the protocol.
	MagNa Mix was improperly handled	Resuspend the MagNa Mix by vortexing prior to use.
	MagNa Mix was disturbed or lost during binding or washing steps	Carefully remove the supernatant from the tube without removing the tube from the MagNa Stand. Make sure that MagNa particles are not disturbed.
	Improper elution	Completely resuspend the MagNa particles in elution buffer before

		incubation at 56°C for elution.
	Ethanol is not added to wash buffers	Add 100% ethanol to wash buffers prior to use as mentioned on the bottles.
Poor performance of extracted DNA in downstream applications	Ethanol carryover	Air dry the MagNa particles properly after washing steps, to remove the ethanol completely, but do not over dry the pellet.
	Salt carryover	Ensure that the correct amount of ethanol added to the Wash Buffers and two wash steps are performed with Wash Buffer 2.