BLOOD DNA EXTRACTION - MIDI KIT

Kit Components

Components	Storage
Blood Lysis Buffer 1	RT
Solution A	RT
Blood Lysis Buffer 2	RT
Proteinase K	2 - 8ºC
Proteinase K Buffer	2 - 8ºC
Blood MagNa Mix	RT
Blood Wash Buffer 1	RT
Blood Wash Buffer 2	RT
Blood Elution Buffer	2 - 8ºC
MagNa Stand (optional)	RT

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 - 8ºC.

PROTOCOL

Sample

- Human blood stored in EDTA\Heparin\Citrate\Fluoride treated vacutainer (Fresh, old or frozen)

Blood Lysate Preparation

1. To a sterile 15 ml centrifuge tube, add **4ml of blood sample**.
2. Add **10 ml of Blood Lysis Buffer 1** and mix the contents by inverting the tube 8 - 10 times.
3. Add **500 µl of Solution A** and mix the contents by inverting the tube 8 - 10 times (*Do not pipette mix*).
4. Incubate at room temperature for 10 minutes.

5. Centrifuge at **8000 rpm for 15 minutes** at room temperature.
6. Carefully discard the supernatant (using pipette) and add **10 ml of Blood Lysis Buffer 1.**

Note: Do not discard supernatant by inverting the tube.

7. Completely resuspend the pellet by pipette mixing.
8. Centrifuge the sample at **8000 rpm for 15 minutes** at room temperature and carefully discard the supernatant using pipette.

   Note: Do not discard supernatant by inverting the tube.
9. Add **5 ml of Blood Lysis Buffer 2** and completely resuspend the pellet by pipette mixing.
10. Add **60 µl of Proteinase K** and mix the contents by pipetting.
11. Incubate at **56°C for 30 minutes.**
12. Transfer the lysate to a fresh 15 ml centrifuge tube.

DNA Binding and Washing

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

13. Add **5 ml of Blood MagNa Mix** to the lysate and mix gently by inverting the tube 10 - 12 times. Incubate at room temperature for 20 minutes.
14. Place the tube on MagNa Stand for 15 minutes.
15. Carefully discard the supernatant without removing the tube from MagNa Stand (*Make sure that the pellet is not disturbed*).
16. Add **5 ml of Blood Wash Buffer 1,** remove the tube from MagNa Stand and resuspend the pellet by pipette mixing for about 10 - 12 times or until complete dispersion of particles.
17. Place the tube back on MagNa Stand until the solution becomes clear.
18. Carefully discard the supernatant without removing the tube from MagNa Stand (*Make sure that the pellet is not disturbed*).
19. Add **5 ml of Blood Wash Buffer 2** and gently invert the tube without removing the tube from MagNa Stand for 5 - 6 times (*Surface wash only*).

20. Discard the supernatant without removing the tube from MagNa Stand.
21. Repeat steps 19 - 20.
22. Air dry the pellet with the tube on MagNa Stand at room temperature for 20 - 30 minutes.

Note: *Do not over dry the pellet.*

DNA Elution

23. After drying, remove the tube from MagNa Stand.
24. Add **1 ml of Elution Buffer** and resuspend the pellet by pipette mixing 10 – 12 times (*Ensure complete dispersion of particles*).
25. Incubate at **56ºC for 10 minutes** with intermittent tapping.
26. Place the tube back on MagNa Stand for 5 minutes or until solution gets cleared.
27. Carefully transfer the supernatant containing DNA to a sterile 1.5 ml microcentrifuge tube without removing the tube from MagNa Stand. Make sure that the pellet is not disturbed.

Optional: *To ensure complete recovery of pure DNA, a second elution can be performed in 250µl of elution buffer.*

Note: In the elution step, if the MagNa particles take more than 10 minutes for clearing, then spin the tubes at 14,000 rpm for 5 minutes and place the tubes back on MagNa Stand until solution becomes clear. Transfer the supernatant to a fresh tube.

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	Use suggested amount of Proteinase K for the specified time.
		Ensure proper re-suspension of pellet while adding the 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 as 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 without disturbing the MagNa particles.
	Improper elution	Completely resuspend the MagNa particles in elution buffer before incubation at 56^{o}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.