Xpress Total Nucleic Acid Kit

Protocol for isolation of high quality total Nucleic acid from whole blood EDTA\Heparin\ Citrate\Fluoride treated vacutainer (fresh/frozen/stored).

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Lysis Buffer	RT	RT
Solution X	RT	RT
Proteinase K & Buffer	2 - 8 °C	RT
Carrier RNA & Buffer	- 20 °C	RT
MagNa Mix	RT	RT
Wash Buffer 1	RT	RT
Wash Buffer 2	RT	RT
Elution Buffer	RT	RT
MagNa Stand (optional)	RT	RT

* RT denotes 15 - 25°C.

Materials not provided with the kit

1. 100% Ethanol to Wash Buffers as indicated on the bottle.
2. Water bath/heat block at 65°C and 56°C.
3. Reconstitute Proteinase K with Proteinase K dilution Buffer and store at 2 – 8°C.
4. Reconstitute Carrier RNA with Carrier RNA dilution Buffer and store at – 20 °C.

Important

Use standard lab practices and follow the safety information prior to using the kit. Refer to the Material Safety Data Sheet (MSDS) on our website.

Technical Support

Email: support@maggenome.com

Blood Lysis & Binding	1. To a sterile 1.5 ml tube, add 200 µl of sample. 2. Add 30 µl of Solution X and quick vortex. 3. Add 300 µl of Lysis Buffer and quick vortex. 4. Add 20 µl of Proteinase K and quick vortex. 5. Add 5 µl of Carrier RNA and quick vortex *(Note: Vortex the MagNa Mix thoroughly before the next step)* 6. Add 300 µl of MagNa Mix and quick vortex. 7. Short spin the constituents. 8. Incubate at 65°C for 30 minutes. 9. Place the tube on MagNa Stand and incubate the samples at RT for 5 minutes. 10. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.

DNA Washing	11. To the magnetic nanoparticles, add 750 µl of Wash Buffer 1 & gently invert mix the tube 5-6 times, without removing it from the MagNa Stand *(surfacewash only).* 12. Incubate the tube for 1 minute until solution appears clear. 13. Carefully discard the supernatant without removing the tube from the MagNa Stand Ensure the magnetic nanoparticles are not disturbed. 14. To the magnetic nanoparticles, again add 750 µl of Wash Buffer 1, remove the tube from the MagNa Stand and resuspend by thorough pipette mixing to ensure complete dispersion of the particles. *(Note: Use 200 µl pipette for better resuspension of the pellet)* 15. Place the tube back on the MagNa Stand for 30 - 60 seconds till the solution becomes clear. 16. Discard the supernatant without removing the tube from the MagNa stand. Ensure the magnetic nanoparticles are not disturbed. 17. Add 750 µl of Wash Buffer 2 & gently invert mix the tube 5 – 6 times without removing from the MagNa Stand *(surface wash only).* 18. Discard the supernatant without removing the tube from the MagNa Stand. 19. Repeat steps 17 -18. 20. Air dry the magnetic nanoparticles without removing the tube from MagNa Stand for 5-10 minutes without over drying them.

DNA Elution	21. After drying, remove the tube from the MagNa Stand. 22. Add 50 µl of Elution buffer and resuspend the magnetic nanoparticles by pipette mixing thoroughly. 23. Incubate at 56°C for 5minutes with intermittent tapping. 24. Place the tube back on the MagNa Stand for 5 min. or until the solution becomes clear.

	25. Carefully transfer the supernatant containing Nucleic acid to a sterile 1.5 ml tube, without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed. 26. Discard the magnetic nanoparticles in the appropriate hazard container.

Note: In the elution step, if the Magnetic particles take more than 10 minutes for clearing, spin the tubes at 14,000 rpm for 5 minutes, place on MagNa Stand until solution clears and then collect the supernatant with pure nucleic acid.

Troubleshooting Guide

Observation	Possible causes	Suggested Solution
Low DNA yield or Poor Quality	IncompleteLysis	Use the suggested Proteinase K volume for complete lysis.
		Performing quick vortex after addition of each buffer to be performed.
		Make sure that the incubation temperature and time for lysis/ elution is followed as per the protocol.
	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.
	Magnetic particle loss during binding or washing steps	Carefully remove the supernatant from the tube without removing the tube from the magnetic stand and without disturbing the MagNa particles.
	Improper elution	Completely resuspend the MagNa particles in elution buffer before incubation at 56°C for elution. Tap the tube few times during the 5 min incubation.
	Ethanol is not added to wash buffers	Add 100%ethanol to wash buffers before use as indicated on the bottles.
Poor performance of extracted DNA in downstream applications	Ethanol carryover	Air dry the MagNa particles after the washing steps to remove ethanol completely, but do not over dry the pellet.
	Salt carryover	Ensure that the correct amount of ethanol is added to the Wash Buffers and the two wash steps are performed with Wash Buffer 2.