XpressDNA Pathogen Blood Kit *(protocol for extraction from blood medium)*

Protocol for isolating high quality total genomic DNA from a wide variety of fungi, gram positive and gram-negative bacterial species from blood samples using XpressDNA Pathogen kit.

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Pathogen Lysis Buffer 1	RT	RT
Solution A	RT	RT
Pathogen Enzyme Buffer	RT	RT
Pathogen Lysis Buffer 2	RT	RT
Proteinase K	2 - 8 °C	RT
Proteinase K Buffer	2 - 8 °C	RT
RNase A	2 - 8 °C	RT
Pathogen MagNa Mix	RT	RT
Pathogen Wash Buffer 1	RT	RT
Pathogen Wash Buffer 2	RT	RT
Pathogen 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 56°C and 37°C
3. Reconstitute Proteinase K with Proteinase K Buffer and store at 2 - 8°C.
4. Lysozyme stock (20 mg/ml), to be prepared freshly in molecular biology grade water.

Important

Pay attention to standard lab practices and safety information before beginning the procedure. For more information, refer the appropriate Material Safety Data Sheet (MSDS) available from the product supplier or download from our website http://www.maggenome.com/

Technical Support

For any product related queries please write to us on info@maggenome.com, sales@maggenome.com, support@maggenome.com.

Protocol

Blood Lysate Preparation	1. To a sterile 1.5 ml microcentrifuge tube, add **400 µl of blood sample.** 2. Add **1 ml of Pathogen Lysis Buffer 1** and mix the contents by inverting the tube 4 - 5 times. 3. Add **50 µl of Solution A** and mix the contents by inverting the tube 4 - 5 times *(do not pipette mix).* 4. Incubate at room temperature for 5 minutes. 5. Centrifuge at 8000 rpm for 2 minutes at room temperature. 6. Carefully discard the supernatant and add **1 ml of Pathogen Lysis Buffer 1.** 7. Resuspend the pellet in the buffer *(thoroughly by pipette mixing).* 8. Centrifuge the sample at 8000 rpm for 2 minutes at room temperature and carefully discard the supernatant. 9. To the pellet, add **190 µl of Pathogen Enzyme Buffer** and completely resuspend the pellet by pipette mixing *(ensure proper resuspension of pellet).* 10. Add **30 µl of freshly prepared lysozyme** and mix thoroughly by pipette *(pipette mixing 3 - 4 times).* 11. Incubate at 37°C for 30 minutes. 12. Add **800 µl of Pathogen Lysis Buffer 2** and mix thoroughly by pipette *(pipette mixing 10 - 15 times).* 13. Add **20 µl of Proteinase K** and quick vortex. 14. Incubate at 56°C for 20 minutes. *(Pipette mix completely every 30 minutes).* *Note: For better dispersion, use 200 µl pipette for re-suspending the pellet* 15. Add **10 µl of RNase A** and quick vortex. 16. Incubate at room temperature for 10 minutes. 17. Centrifuge at 14,000 rpm for 5 minutes at room temperature. 18. Transfer the supernatant to a fresh 1.5 ml tube.

DNA Binding	*(Note: Vortex the Blood MagNa Mix thoroughly before the next step)* 19. Add **400 µl of Pathogen MagNa Mix** and gently invert the tube 10 - 12 times to mix properly. Do not vortex. 20. Incubate the samples at RT for 5 minutes. 21. Place the tube on a MagNa Stand until the solution becomes clear. 22. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.

DNA Washing	23. To the magnetic nanoparticles, add **500 µl of Pathogen 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.)* 24. Place the tube back on the MagNa Stand for 30 - 60 seconds till the solution becomes clear. 25. Discard the supernatant without removing the tube from the MagNa stand. Ensure the magnetic nanoparticles are not disturbed. 26. Add **500 µl of Blood Wash Buffer 2** & gently invert mix the tube 5 – 6 times without removing from the MagNa Stand *(surface wash only).* 27. Discard the supernatant without removing the tube from the MagNa Stand. 28. Repeat steps **26 - 27.** 29. Air dry the magnetic nanoparticles without removing the tube from MagNa Stand for 10 - 15 minutes without over drying them.

DNA Elution	30. After drying, remove the tube from the MagNa Stand. 31. Add **50 - 100 µl of Blood Elution buffer** and resuspend the magnetic nanoparticles by pipette mixing thoroughly. 32. Incubate at 56°C for 5 minutes with intermittent tapping**.** 33. Place the tube back on the MagNa Stand for 5 minutes or until the solution becomes clear. 34. Carefully transfer the supernatant containing DNA to a sterile 1.5 ml tube, without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed. 35. 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 DNA.

Troubleshooting Guide

Observation	Possible causes	Suggested Solution
Low DNA yield or Poor Quality	Incomplete Lysis	Proteinase K was not used at the suggested amount and for the specified time.
		Ensure proper re-suspension of the pellet while adding the Lysis buffers.
		Make sure that the incubation temperature and time for lysis is followed as per the protocol.
		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.
	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 10 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.