XpressDNA Stool Kit

Protocol for isolation of high quality total genomic DNA from stool samples (fresh/frozen/stool samples stored in stabilization/storage buffers).

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Solution F1	RT	RT
Solution F2	RT	RT
Solution F3	RT	RT
Solution F4	RT	RT
Stool MagNa Mix	RT	RT
Stool Wash Buffer 1	RT	RT
Stool Wash buffer 2	RT	RT
Stool 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 65°C.

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

Lysis of stool sample **(sample stored in storage/ stabilization buffer)**	1. Add **400 µl of Stool sample** stored in storage/ stabilization buffer to a 1.5 ml tube. 2. Incubate at 65°C for 20 minutes. 3. Proceed to step 7.
Lysis of stool sample ***(Solid Sample)***	4. Add **50 - 100 mg of Stool sample** to 1.5 ml screw cap tube. *(For fecal samples that are especially high in lipids, polysaccharides and protein (e.g. meconium or some bird feces) smaller amounts of starting material may improve DNA yield and purity.)* 5. Add **400 µl of Solution F1** and vortex for 1 minute. 6. Incubate at 65°C for 20 minutes ***(with intermittent vortex mixing).*** 7. Add **400 µl of Solution F2** and vortex for 1 minute. 8. Add **100 µl of Solution F3** and vortex for 1 minute. 9. Centrifuge the tubes at 14500 rpm for 3 minutes**.** 10. Transfer **650 µl of the supernatant** to a 1.5 ml tube. 11. **Add 300 µl of Solution F4** and vortex for 15 seconds. Incubate on ice for 10 minutes. 12. Centrifuge the tubes at 14500 rpm for 5 minutes. 13. Transfer **750 µl of the supernatant** to a clean 1.5 ml tube.

DNA Binding	***(Note: Vortex the MagNa Mix thoroughly before the next steps)*** 14. Add **400 µl of Stool MagNa Mix** and invert mix the tube 8 - 10 times. Do not vortex. 15. Incubate the samples at RT for 5 minutes. 16. Place the tube on a MagNa Stand for 30 - 60 seconds or until the solution becomes clear. 17. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.

DNA Washing	18. Add **1 ml of Stool Wash Buffer 1** and remove the tube from the MagNa Stand. 19. Resuspend the magnetic particles by pipette mixing thoroughly to ensure complete dispersal of the particles. 20. Place the tube back on the MagNa Stand for 30 - 60 seconds until the solution becomes clear. 21. Discard the supernatant without removing the tube from the MagNa Stand. ***(Note: Make sure the magnetic particles are not disturbed)*** 22. Add **750 µl of Stool Wash Buffer 2** and gently invert mix the tube 5 - 6 times without removing from the MagNa Stand ***(surface wash only).*** 23. Discard the supernatant without removing the tube from the MagNa Stand. 24. Repeat steps **22 and 23**. 25. Air dry the magnetic nanoparticles without removing the tube from the MagNa Stand for 10 - 15 minutes. Avoid over drying.

DNA Elution	26. After drying, remove the tube from the MagNa Stand. 27. Add **50 µl of elution buffer**. Resuspend the magnetic nanoparticles by pipette mixing thoroughly. 28. Incubate at **56°C for 5 minutes** with intermittent tapping. 29. Place the tube back on the MagNa Stand for 5 minutes or until the solution becomes clear. 30. 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. 31. Discard the Magnetic particles in the appropriate hazard container.

Note: In the elution step, if the magnetic nanoparticles 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	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 nanoparticle loss during binding or washing steps	Carefully remove the supernatant from the tube without removing the tube from the MagNa Stand and without disturbing the Magnetic nanoparticles.
	Improper elution	Completely resuspend the Magnetic 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	Inhibitor carryover	Use exact volume of solution F4 for efficient removal of inhibitors.
	Ethanol carryover	Air dry the Magnetic 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 two wash steps are performed with Wash Buffer 2.