XpressDNA Water Kit

Protocol for isolation of high quality total microbial DNA from a variety of water samples.

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Solution W1	RT	RT
Solution W2	RT	RT
Solution W3	RT	RT
Solution W4	RT	RT
Solution W5	RT	RT
Water MagNa Mix	RT	RT
Water Wash Buffer 1	RT	RT
Water Wash Buffer 2	RT	RT
Water 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.

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.

Pretreatment:

A. Filter the water sample using a Whatman grade 1 Filter membrane. The volume of water filtered will depend on the microbial load and turbidity of the water sample.

Note: Please see Table 1: Types of Water (For Volume)

B. Carefully separate the filter membrane from the base and place (sample collected side "up") into a sterile Petri dish.

C. Cut the pellets collected section of membrane into small (2-5 mm) pieces.

Note: If the water is highly turbid. Centrifuge 10-15ml the water sample at 14500rpm for 5mins to collect the pellets only.

Table 1:	
Type of water sample	**Sample volume (ml)**
Saltwater bay	≅ 500ml-1L
Freshwater lake	≅ 1L-1.5L
brackish water	≅ 1L-1.5L
Ocean coastal	≅ 500ml-1L
Sewage influent	≅ 500ml-1L
Treated effluent	≅ 500ml-1L

Protocol

Soil lysate Preparation	1. Add **required volume of collected pellets** (0.3g-1g) to 2 ml screw cap tube. 2. Add **500 µl of solution W1** and vortex for 30 seconds. 3. Add **100 µl of solution W2.** 4. Place the tubes horizontally on the vortexes. *(Note: Fasten the tubes properly during vortexing and make sure the contents of the tubes are shaken vigorously.)* 5. Vortex at maximum speed for 20 minutes. 6. Add **250 µl of solution W3.** Place the tube horizontally on the vortex and vortex for 10 minutes**.** 7. Centrifuge the tubes at 14000 rpm for 5 minutes. 8. Transfer **650 µl of the supernatant** to a 1.5 ml tube. 9. Add **300 µl of solution W4** and vortex for 5 seconds. Incubate on ice for 10 minutes. 10. Centrifuge the tubes for 5 minutes at 14500 rpm. 11. Transfer **750 µl of the supernatant** to a clean 1.5 ml tube. 12. Add **200 µl of solution W5** and vortex for 5 seconds. Incubate on ice for 10 minutes. 13. Centrifuge the tubes for 5 minutes at 14500 rpm. 14. Transfer **750 µl of the supernatant** to a clean 1.5 ml tube.

DNA Binding	*(Note: Vortex the water MagNa Mix thoroughly before the next step)* 15. Add **350 µl of water MagNa Mix** to the supernatant. 16. Gently invert mix the contents 10 – 12 times. Do not vortex. 17. Incubate at RT for 5 minutes. 18. Place the tube on a magnetic stand for 5 minutes or until the solution becomes clear. 19. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.

Washing	20. **Add 1 ml of water Wash Buffer 1** and remove the tube from the MagNa Stand. 21. Resuspend the magnetic nanoparticles by pipette mixing thoroughly to ensure complete dispersion. 22. Place the tube back on the MagNa Stand for 30 - 60 seconds till the solution becomes clear. 23. Carefully discard the supernatant with the tube on the MagNa stand. Ensure the magnetic nanoparticles are not disturbed. 24. Add **750 µl of water Wash Buffer 2** and gently invert mix the tube 5 - 6 times without removing it from the MagNa Stand *(surface wash only).* 25. Discard the supernatant without removing the tube from the MagNa stand. 26. Repeat steps **24 and 25**. 27. Air dry the magnetic nanoparticles without removing the tube from the MagNa Stand for 10 - 12 minutes. Avoid over drying.
DNA Elution	28. After drying, remove the tube from the MagNa Stand. 29. Add **50 µl of water elution buffer** and resuspend the magnetic nanoparticles by pipette mixing thoroughly. 30. Incubate at 56°C for 10 minutes with intermittent tapping. 31. Place the tube back on the MagNa Stand for 5 minutes or until the solution becomes clear. 32. 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. 33. Discard the magnetic nanoparticles 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.

XpressDNA Water Kit Highlights

• Sample types: Ocean water, fresh water, brackish water, ground water, tap water, lake water, river water, sewage water.

• Reliable – Reproducible DNA purification from variety of sample sources.

• High quality – Inhibitor free DNA compatible with common downstream applications such as PCR and next-generation sequencing.

• High yield and purity – Efficient purification of DNA from even specialized samples.

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.
	Sample -improper storage /poor microbial content	Increase the initial amount of water sample based on the water type and microbial content.
	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 nanoparticles in elution buffer before incubation at 56°C for elution. Tap the tube few times during the 10 minute 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 W4 and W5 for efficient removal of inhibitors.
	Ethanol carryover	Air dry the magnetic nanoparticles 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.