XpressDNA Bacteria Kit

Protocol for isolation of high quality total genomic DNA from both gram negative and gram positive bacteria.

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Bacteria Enzyme Buffer	RT	RT
Bacteria Lysis Buffer	RT	RT
Proteinase K	2 - 8 °C	RT
Proteinase K Buffer	2 - 8 °C	RT
RNase A	2 - 8 °C	RT
Bacteria MagNa Mix	RT	RT
Bacteria Wash Buffer 1	RT	RT
Bacteria Wash Buffer 2	RT	RT
Bacteria 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.
3. Reconstitute Proteinase K in Proteinase K Buffer and store at 2 – 8°C.
4. 1X PBS
5. 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 with 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.

Sample Preparation

1. Take 1 ml of overnight grown bacterial culture (OD_{600} > 1) in a 1.5 ml tube and centrifuge at 10,000 rpm for 5 minutes at RT. Discard supernatant.
2. Add 500 µl of **1X PBS** and resuspend the pellet completely by pipette mixing.
3. Centrifuge at 10,000 rpm for 5 minutes at RT and discard the supernatant.

For Gram Positive bacteria proceed to step 4.

For Gram Negative bacteria proceed to step 14.

Protocol

Gram Positive Lysate Preparation	4. To the pellet, add 190 µl of **Bacteria Enzyme Buffer** and completely resuspend the pellet by pipette mixing. 5. Add **10 µl of freshly prepared lysozyme** and perform thorough pipette mixing. 6. Incubate at 37°C for 60 minutes. 7. Add **800 µl of Bacteria Lysis Buffer** and perform thorough pipette mixing. 8. Add **20 µl of Proteinase K** and perform quick vortex. 9. Incubate at 56°C for 60 minutes, pipette mixing the suspension thoroughly every 30 minutes. *(Note: For better dispersion, use 200 µl pipette for re-suspending the pellet)* 10. Add **10 µl of RNase A** and vortex the tube for 30 seconds. 11. Incubate at RT for 15 minutes. 12. Centrifuge at 14,000 rpm for 5 minutes at RT. 13. Transfer the supernatant to a fresh 1.5 ml tube. **Proceed to step 21 (DNA binding and washing)**

Gram Negative lysate preparation	14. Add **1 ml of Bacteria Lysis buffer** to the pellet and resuspend the pellet by pipette mixing *(10 – 15 times).* 15. Add **20 µl of Proteinase K** and perform quick vortex. 16. Incubate at 56°C for 60 minutes, pipette mixing the suspension thoroughly every 30 minutes. *(Note: For better dispersion, use 200 µl pipette for re-suspending the pellet)* 17. Add **10 µl of RNase A** and vortex the tube for 30 seconds. 18. Incubate at RT for 15 minutes. 19. Centrifuge at 14,000 rpm for 5 minutes at RT**.** 20. Transfer the supernatant to a fresh 1.5 ml tube. **Proceed to step 21 (DNA binding and washing)**

DNA Binding	*(Note: Vortex the Bacteria MagNa Mix thoroughly before the next steps)* 21. Add **400 µl of Bacteria MagNa Mix** to the supernatant. Gently mix the contents by inverting the tube for 8 - 10 times (Do not vortex). 22. Incubate at RT for 5 minutes. 23. Place the tube on a MagNa Stand at RT for 5 minutes. 24. Carefully discard the supernatant without removing the tube from the MagNa Stand. *(Make sure the magnetic nanoparticles are not disturbed)*

DNA Washing	25. Add **500 µl** of **Bacteria Wash Buffer 1** and remove the tube from the MagNa Stand. 26. Resuspend the Magnetic nanoparticles by pipette mixing 10 - 12 times to ensure complete dispersion of the particles. Do not vortex. *(Note: For better dispersion, use 200 µl pipette for re-suspending the pellet)* 27. Place the tube back on the MagNa Stand and allow it to stand for 30 - 60 seconds until the solution becomes clear. 28. Carefully discard the supernatant without removing the tube from the MagNa Stand. *(Make sure the Magnetic particles are not disturbed).* 29. Add **500 µl of Bacteria Wash Buffer 2** and gently invert mix the tube 5 – 6 times without removing from the MagNa Stand. *(Surface wash only).* 30. Discard the supernatant without removing the tube from the MagNa Stand. 31. Repeat the steps **29 - 30.** 32. Air dry the Magnetic nanoparticles with the tube on the MagNa Stand at RT for 10 – 15 minutes. Avoid over drying.

DNA Elution	33. After drying, remove the tube from the MagNa Stand. 34. Add **50 - 100 µl of Bacteria Elution Buffer** and resuspend the Magnetic nanoparticles by pipette mixing thoroughly. 35. Incubate at **56°C for 10 minutes** with intermittent tapping. 36. Place the tube on the MagNa Stand for 5 minutes or until the solution becomes clear. 37. 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. 38. 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

Troubleshooting Guide

Observation	Possible causes	Suggested Solution
Low DNA Yield or Poor Quality	Low Culture Density	Use bacterial culture with OD_{600} above 1.
		For better yield, use overnight grown cell culture or cell pellets stored in proper conditions.
	Poor Cell Lysis	Cells may not be dispersed properly in lysis buffer. Disperse the cells by adequate pipette mixing.
		For bacterial strains with high DNA content, the lysate may appear viscous. For such samples thorough pipette mixing is recommended.
		Add required volume of Proteinase K into the Lysis buffer. Ensure optimal lysis temperature. Proteinase K solution should be stored at 2 - 8°C.
	Ethanol is not added to wash buffers	Add 100% ethanol to wash buffers as suggested on the bottles.
RNA contamination	RNase A not added	Add RNase A as per the protocol.
Poor performance of extracted DNA in downstream applications	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 the two wash steps are performed with Wash Buffer 2.