XpressDNA Plasmid Kit

Protocol for isolation of highly pure plasmid DNA from bacterial samples.

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
Plasmid Extraction Buffer	2 - 8°C	RT
Plasmid MagNa Mix	2 - 8°C	RT
Plasmid Wash Buffer	2 - 8°C	RT
Plasmid Elution Buffer	2 - 8°C	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 80°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

Bacterial Lysate Preparation	1. Harvest **1.5 ml of overnight bacterial culture** (OD_{600} >1 - 1.5) in a 1.5 ml tube and centrifuge at 10,000 rpm for 5 minutes. Carefully discard the supernatant and remove traces of excess medium, if any. *(Note: For low copy number plasmids, bacterial culture volume can be varied up to 2 ml)* 2. Add **150 µl of Plasmid Extraction Buffer** and gently resuspend the pellet by pipette mixing, until the cells are completely dispersed in the buffer. 3. Incubate at **80°C for 3 minutes**. 4. After incubation, centrifuge the lysate at 14,500 rpm for 10 minutes. 5. Transfer the supernatant to a fresh 1.5 ml tube without disturbing the pellet.
DNA Binding	*(Note: Vortex the MagNa Mix thoroughly before the next step)* 6. Add **120 µl of Plasmid MagNa Mix** to the supernatant and invert mix gently 10 - 12 times. Do not vortex. 7. Incubate at RT for 5 minutes. 8. After incubation, place the tube on a MagNa Stand for 2 minutes or until the solution becomes clear. 9. Discard the supernatant without removing the tube from the MagNa Stand.
DNA Washing	10. To the magnetic nanoparticles, add **200 µl of Plasmid Wash Buffer** and invert mix gently 5 - 6 times without removing the tube from the MagNa Stand. 11. Discard the supernatant without removing the tube from the MagNa Stand. 12. Repeat steps **10 - 11.** 13. Air dry the MNPs the tube on the MagNa Stand at RT for 5 - 10 minutes. Avoid over-drying.
DNA Elution	14. Remove the tube from the MagNa Stand and add **45 µl of Plasmid Elution Buffer.** 15. Carefully resuspend the pellet by gentle pipette mixing. 16. Incubate the tube at 80°C for 2 - 3 minutes with intermittent tapping. 17. After incubation, place the tube on the MagNa Stand until the solution becomes clear. 18. 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. 19. Discard the magnetic nanoparticles in the appropriate hazard container.

Note: In the elution step, if magnetic nanoparticles take more than 10 minutes to clear, spin the tube at 14,000 rpm for 5 minutes, place on the magnetic Stand and collect the supernatant containing DNA.

Experimental set up for large culture volumes

Bacterial culture volume	5 ml	10 ml
Extraction Buffer Volume (*step* 2)	500 µl	1200 µl
Incubation @ 80°C (*step* 3)	5 minutes	5 minutes
MagNa Mix volume (*step* 6)	400 µl	800 µl
Wash Buffer volume (*step* 9)	500 µl (twice)	500 µl (twice)
Elution volume (*step* 12)	100 µl	150 µl

Troubleshooting Guide

Observation	Possible causes	Suggested Solution
Low DNA yield or Poor Quality	Insufficient Cell pellet	Take correct volume of bacterial culture.
		Ensure that OD_{600} is above 1 - 1.5.
Genomic DNA contamination	Low copy number plasmids	For low copy number plasmids, bacterial culture volume can be varied up to 2 ml.
	Prolonged incubation time at 80°C during lysis	Ensure that lysis incubation time is followed accurately.
No plasmid DNA	Old bacterial cultures	Perform experiment with overnight culture of bacteria.
	Absence of ethanol in Plasmid Wash Buffer	Ensure that correct volume of 98 - 100% Ethanol is added in the Plasmid Wash Buffer.
Multiple DNA bands in Agarose gel	Bacterial culture does not have plasmid	Check whether the culture contains plasmid. Ensure that the cells are properly transformed.
	Nick formation	Harsh pipette mixing may lead to nick formation. Pipette mix gently.
	Plasmid Contamination	Ensure that the cells are properly transformed with single plasmid only.