PLASMID DNA EXTRACTION KIT

Kit Components

Components	Storage
Plasmid Extraction Buffer	2 - 8°C
Plasmid MagNa Mix	RT
Plasmid Wash Buffer	RT
Plasmid Elution Buffer	2 - 8°C
MagNa Stand (optional)	RT

Materials needed prior to start

- Add 96 - 100% Ethanol to the Wash Buffer as indicated on the bottle.
- Set the water bath at 80°C.

PROTOCOL

Bacterial lysate Preparation

1. Harvest 1.5 ml of overnight bacterial culture (OD_{600} >1 - 1.5) by centrifuging 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 upto 2 ml.*

2. Add **150 µl 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 microcentrifuge tube without disturbing the pellet.

Plasmid DNA Binding and Washing

Note*: Vortex the Plasmid MagNa Mix to ensure complete dispersion of the particles.*

6. Add **120 µl of Plasmid MagNa Mix** to the supernatant and mix gently by inverting the tube 10 - 12 times. Incubate at room temperature for 5 minutes.

7. After incubation, place the tube on the MagNa Stand for 2 minutes or until the solution becomes clear.
8. Decant the supernatant without removing the tube from MagNa Stand.
9. Add **200 µl of Plasmid Wash Buffer** and invert mix gently 5 - 6 times without removing the tube from MagNa Stand.
10. Decant the supernatant without removing the tube from MagNa Stand.
11. Repeat step 9 - 10.
12. Air dry the pellet without removing the tube from MagNa Stand for 5 - 10 minutes.

Note: Do not over dry the pellet.

Plasmid DNA Elution

13. Remove the tube from the MagNa Stand and add **45 µl of Plasmid Elution Buffer.**
14. Carefully resuspend the pellet by gentle pipette mixing. *(Do not vortex the pellet).*
15. Incubate the tube at **80°C for 2 - 3 minutes** with intermittent tapping.
16. After incubation, place the tube on MagNa Stand until the solution becomes clear.
17. Transfer the supernatant containing the plasmid to a fresh microcentrifuge tube without disturbing the pellet.

Note: In the elution step, if MagNa particles take more than 10 minutes for clearing, then spin the tubes at 14,000 rpm for 5 minutes, place on MagNa Stand and collect the supernatant for pure 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)	1000 µl	1000 µl
Elution volume (*step* 12)	100 µl	150 µl

Downstream applications

Plasmid DNA obtained from this method is ready to use for any downstream application without any further precipitation or purification step.

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.
	Low copy number plasmids	For low copy number plasmids, bacterial culture volume can be varied up to 2 ml.
Genomic DNA contamination	Prolonged incubation time at 80°C during lysis	Ensure that lysis incubation time is followed accurately.
	Old bacterial cultures	Perform experiment with overnight culture of bacteria.
No plasmid DNA	Absence of ethanol in Plasmid Wash Buffer	Ensure that correct volume of 98 - 100% Ethanol is added in the Plasmid Wash Buffer.
	Bacterial culture does not have plasmid	Check whether the culture contains plasmid. Ensure that the cells are properly transformed.
Multiple DNA bands in Agarose gel	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.