

Plasmid DNA Extraction and Purification

#Cat : NB-26-01728

size : 100Tests

Kit Components included:	• Si-Mag magnetic beads – 1.5 ml • RNase A solution – 60 ul • Suspension solution – 20 ml • Lysis solution – 20 ml • Neutralization solution – 35 ml • Wash solution – 25 ml • Elution Buffer – 10 ml
Materials needed but not provided with the kit:	• 80% Ethanol • Si-Mag Magnet (sold separately) or other magnetic racks compatible with vials used. • Isopropanol (ACS grade).
Applications:	This kit provides a simple, rapid and efficient method for the recovery and purification of DNA directly from Agarose gel (100 bp to 50 kb) with typical recovery efficiency up to 85%.
Storage:	Magnetic beads should be stored at 2-8°C but other kit reagents need to be stored at room temperature. Lysis solution may turn cloudy if store in the cold room and to clear it up place the bottle into a water bath at 37°C.

Introduction

This kit, with proprietary separation and buffer systems, allows extracting and purifying plasmid DNA from 2.0~5.0 mL of bacteria culture. The typical yield is 10~20 µg of high quality plasmid DNA, and the typical OD_{260/280} is between 1.75 and 1.85.

Purified plasmid DNA can be directly used for a variety of molecular biology applications such as enzymatic digestion, sequencing and transformation.

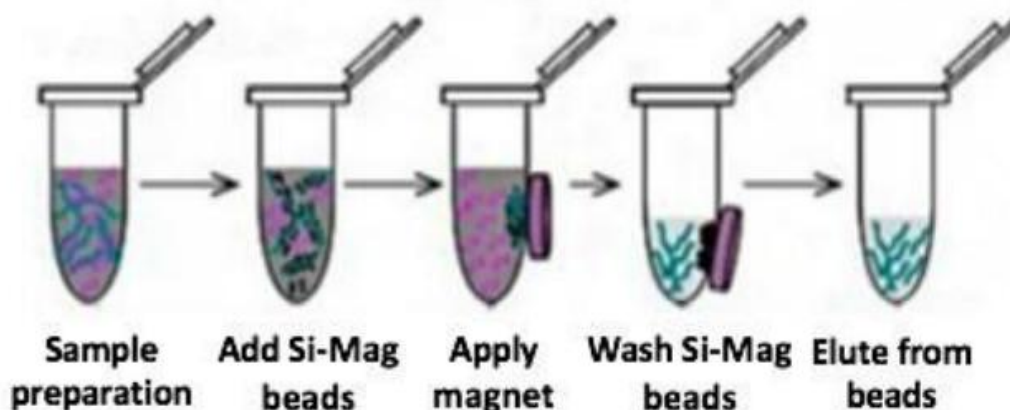
The kit will work with a 48 well round bottom plates if a special magnetic frame is used.

The kit can also be used with a variety of automatic nucleic acid extraction instruments or workstations.

Precautions

- Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.
- Avoid freeze/thaw cycles and centrifugation which could damage the beads.
- Vortex samples for about 10 seconds before adding magnetic beads.
- Vortex beads for about 10 seconds and mix them well with DNA containing material to ensure best performance.
- Elute DNA from the beads completely.

Principle of Assay:



Procedure for purification of plasmid DNA from bacterial culture

1. Preparation. Before the first use, add all the RNase A solution into the Suspension Solution.
2. Harvest cells. Spin 1.5-5 mL of overnight grown bacterial cells at 12,000 rpm for 2 min.
3. Re-suspend the cells. Remove culture media completely. Add 200 μ L of suspension solution (with RNase A), then vortex to ensure complete suspension of cell. Transfer cells to a clean Eppendorf tube.
4. Lyse the cells. Add 200 μ L of Lysis solution and mix by inverting the tube for 6-8 times, then incubate for 2 min. Do NOT vortex.
5. Neutralize the solution. Add 350 μ L of Neutralization solution and mix by inverting the tube for 8-10 times. Do NOT vortex.
6. Spin the tube at 12,000 rpm for 15 min at 4°C.
7. Transfer the solution to a clean Eppendorf tube, then add 15 μ L of magnetic beads, mix well and incubate 3-5 min at RT. Put Eppendorf tube onto the Si-Mag magnet rack for 20 seconds.
8. Remove solution by holding the magnet rack upside down or by pipetting.
9. Wash the beads with 500 μ L of Wash Solution and then repeat Step 8.
10. Wash the beads with 500 μ L of 80% ethanol twice repeating Step 8.
11. Dry the beads at 55°C for 8 min leaving the tube open. Do not over-dry the beads.
12. Elute the DNA from beads with 50-100 μ L of elution buffer, incubate for at least 2 min and then vortex at full speed for 1 min. Alternatively, incubation at 60°C for 2 min may improve the recovery for DNA larger than 10 kb.
13. Remove beads by using magnet rack, pipette DNA out and transfer to a clean tube.
14. Store purified DNA at -20°C for long-term storage.

FOR RESEARCH USE ONLY

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