SciPhi™

PRODUCT INFORMATION

Plasmid Miniprep Kit

#NXG993

Read Storage and Reagent preparation information before first use.

For Research Use Only. Not recommended for diagnostic use.

Kit Components:

Component	Quantity
Purification Columns with Collection Tubes	50
Collection Tubes (2 ml)	50
Resuspension Solution	15 ml
Lysis Solution	15 ml
Neutralization Solution	20 ml
Wash Buffer (Conc.)	20 ml
RNase A	0.15 ml
Ethanol	40 ml
Elution Buffer	4 ml

Storage Instructions:

SciPhi™ Plasmid Miniprep Kit can be stored at room temperature (15 to 25°C) for upto 12 months. However, the kit can be kept at 4°C in case of storage periods is more than 1 year.
Please remember to properly close the bag containing SciPhi™ Genomic DNA Purification Columns after each usage.

Product Description:

SciPhi™ Plasmid Miniprep Kit is suitable for fast and efficient Plasmid DNA isolation from recombinant *E. coli* cultures. This kit is based on spin column silica membrane technology, eliminating the need of phenol/chloroform extractions. It can isolate Plasmid DNA in less than 15 minutes with a yield of 20 µg. The actual plasmid yield and ideal culture volume rely on the copy number of the plasmid and the cultured medium used. The resulting pure plasmid DNA is instantly ready for utilization across a range of molecular biology techniques, including restriction enzyme digestion, PCR, transformation, and automated sequencing.

Principle:

Resuspension and Lysis: Bacterial cells in pellet form are revitalized and exposed to SDS/alkaline lysis to free the plasmid DNA.

Neutralization: The resulting suspension's pH is adjusted to neutral, providing the best environment for plasmid DNA attachment to the silica membrane of the column.

Removal of cell debris and Binding: Cell debris and SDS precipitate are then pelleted by centrifugation, enabling the efficient binding of plasmid DNA present in supernatant, with silica membrane of the column.

Washings: The bound plasmid DNA undergoes a purification/washing process with wash buffer to effectively remove impurities, ensuring the isolation of high-quality plasmid DNA.

Elution: The purified plasmid DNA is then eluted using an Elution Buffer.

Important Information

a) Buffer Preparation and Handling

i. Incorporate the supplied **RNase A solution** into the **Resuspension Solution**, mix it thoroughly and always store at 4°C. This RNase A added Resuspension solution can remain stable up to 6

months at 4°C.

ii. Prepare **Wash Buffer** according to Table 2 before first use:

Table 1: Instruction to prepare wash buffer:

	Wash Buffer I
Concentrated Wash Buffer	20 ml
Ethanol (96-100%)	35 ml
Total Volume	**55 ml**

Once the ethanol has been added, tick the box on the bottle's cap to indicate that the step has been completed.

iii. Always check the lysis solution and neutralization solution for the presence of any precipitate before each use. If precipitation observed, warm both the solutions at 37°C to redissolve the precipitate. Bring the solutions at room temperature before use.

iv. Handle **Lysis solution** and **Neutralization** solution after wearing the gloves as these buffers contains irritants and may cause harmful effects on skin if not handled properly.

v. Do not add bleach to sample waste container as components present in kit solution may react with the bleach.

vi. There is different wash buffer for EndA+ strains. Please **contact us** to procure same.

b) Growth of Bacterial Cultures:

i. Inoculate 1-5 mL of LB medium supplemented with the appropriate selection antibiotic with a single colony from a newly streaked selective plate. Incubate at 37°C for 12-16 hours with a shaking speed of 200-250 rpm.

ii. Pellet down the culture by centrifugation for 2 minutes at room temperature at 8000 rpm (6800 g) in a microcentrifuge and discard the supernatant. Remove any residual medium from the supernatant by pipette.

c) Recommendations for high-copy-number and low-copy number plasmid isolation:

i. **High-copy-number Plasmids:** It is recommended to **not** use more than 5 ml of culture for plasmid isolation from high-copy-number plasmids.

ii. **Low-copy-number Plasmids:** It is recommended to use up to **10 ml** culture for plasmid isolation from low-copy-number plasmid to recover good yield of plasmid DNA.

Table 2: Copy number of various plasmids

Copy Number	**Number of copies per cell**	**Plasmid Name**
Very low	Up to 5 copies per cell	pSC101 and derivatives
Low-copy	10-50 copies per cell	pBR322 and derivatives
		pACYC and derivatives
High-copy	**300-700 copies per cell**	pUC vectors
		pBluescript vectors
		pGEM vectors
		pTZ vectors
		pJET vectors

Additional Instruments and Consumables Required

- Pipettes
- Sterile pipette tips
- Vortex Mixture
- Microcentrifuge
- Disposable Gloves
- 1.5 ml Microcentrifuge tubes

PROTOCOLS FOR PLASMID DNA ISOLATION

- Carry out all the step of Plasmid DNA isolation at room temperature.
- Perform all centrifugation steps at >12000 x g (10000 – 14000 rpm) in a table-top centrifuge.

1. Pellet down the bacterial cell as recommended in "Important Information" on page no. 5 in **section b(ii)**.
2. Resuspend the cells in 250 µl Resuspension Solution either by pipetting or vortexing to completely remove the clumps. Transfer the prepared cell suspension to a microcentrifuge tube.

   Note: Ensure that RNase A has been added to Resuspension Solution.
3. Dispense 250 µl of the Lysis Solution into the suspension and thoroughly mix the suspension by gently inverting the tube approximately 4 to 6 times until the solution attains a viscous consistency and a clear appearance.

   Note: Use of a vortex mixer is not recommended for mixing as vortexing may lead to shearing of chromosomal DNA. Additionally, avoid allowing the mixture to incubate for more than 5 minutes to avoid denaturation of supercoiled plasmid DNA.
4. Add 350 µl of Neutralization Solution, mix immediately but gently by inverting the tube 4-6 times. The neutralized cell lysate will become cloudy in appearance.

   Note: It is crucial to ensure complete and gentle mixing subsequent to the addition of the Neutralization Solution in order to prevent the localized precipitation of bacterial cell debris.
5. Centrifuge the cell lysate for 5 minutes to pellet down the debris.
6. Carefully transfer the supernatant to purification column by pipetting without disturbing the white precipitate.

   Note: Remember to tightly seal the bag of SciPhi™ Purification Columns tightly after each use!
7. Centrifuge the column for 1 minute. Discard the flow through and place the column in same collection tube.
8. **Optional:** This step is recommended when Plasmid DNA need to be isolated from EndA+ strains.

   Add 500 µl of washing buffer for EndA+ strains to the column and centrifuge for 30-60 seconds. Discard the flow-through.
9. Add 500 µl of Wash Buffer (with ethanol added) to the column followed by centrifugation for 30-60 seconds. Discard the flow-through and place the purification column back into the collection tube.
10. Repeat the step 9 with 500 µl of Wash Buffer.
11. If residual solution remains in the purification column, empty the collection tube and re-spin the column for 1 minute.
12. Discard the collection tube containing the flow-through solution and transfer the column to a new sterile 1.5 ml microcentrifuge tube.
13. Add 50 µl of Elution Buffer to the centre of the Purification Column membrane followed by incubation at room temperature for 2 minutes.

14. After incubation, centrifuge the column for 2 minute to elute the plasmid DNA.

Note

- Consider an extra elution step (optional) using either Elution Buffer or water, as this will help retrieve any remaining plasmid DNA from the membrane and enhance the overall yield by approximately 10-20%.
- In instances where eluting plasmids or cosmids larger than 20 kb, it's advisable to preheat the Elution Buffer to 70°C before applying it to the silica membrane.

15. Discard the purification column. Use the purified plasmid DNA immediately in downstream applications or store at -20 °C until use.

TROUBLESHOOTING

Problem	Possible Cause	Solution
Low yield of plasmid DNA	Old bacterial culture used	Inoculate a freshly isolated individual bacterial colony from an overnight plate into a fresh batch of growth medium containing antibiotics. Allow the cells to grow for a maximum of 16 hours at 37°C with continuous shaking in LB media.
	Incomplete Lysis	• Before lysing, it's crucial to fully resuspend the cell pellet in the Resuspension Solution. Make sure there are no cell clumps visible before adding the Lysis Solution. • Before each use, examine the Lysis Solution for any salt precipitation. If found, redissolve the precipitate by gently warming the solution at 37°C, and then mix it thoroughly prior to usage. • For cell cultivation, it's advisable to use LB media.
	Inefficient elution	Always add the elution buffer to the centre of the column for better elution.
Contaminated Isolated Plasmid	Residual Ethanol	If residual solution is seen in the purification column after washing the column, empty the collection tube and re-spin the column for an additional 1 min.
	RNA Contamination	Ensure hat RNase A was added to the resuspension solution
	Genomic DNA contamination	• To prevent the unintended shearing and release of genomic DNA, avoid using a vortex or shaking the cells during lysis and neutralization. Instead, gently mix the contents by inverting the tube. • Limit the cell lysis duration to no more than 5 minutes.
	Presence of additional band of denatured plasmid	Do not lyse the cells for more than 5 minutes to avoid denaturation.

+91 11 43097949
info@nextgenlife.com
www.nextgenlife.com
NextGen Life Sciences Pvt. Ltd.
F 44-45, Pankaj Central Market,
I. P. Extension, Patparganj, Delhi - 110 092