Cyrusbioscience

GeneKlean® Plasmid Maxiprep Kit

Cat. Number: MAXIP

Storage: Store at Room Temperature.

Sample: 200mL of bacteria cells

Yield: Up to 850ug plasmid

Kit Contents

GeneKlean Plasmid Maxiprep Kit	10 Reactions
GeneKlean® Plasmid Maxiprep Column	10pcs
Solution 1	85mL
Solution 2	85mL
Solution 3	125mL
Wash Buffer 1	105mL
Wash Buffer 2	25mL
(add Ethanol)	(100mL)
Elution Buffer	30mL
RNase A(50mg/mL)	200uL
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Note

- Add provided RNaseA to Solution 1, mix well, store at 4ºC.(Samples Solution 1 already added)
- Add ethanol (>99.5%) to Wash Buffer 2, shake before use (see bottle label for volume).
- Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37˚C.
- Buffers contain irritants. Wear gloves when handling these buffers.

Additional requirements

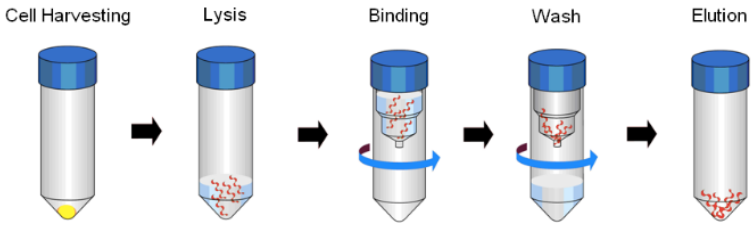
- Ethanol (>99.5%)
- 50mL centrifuge tubes

Description

GeneKlean® Plasmid Maxiprep Kit provides a fast, simple, and cost-effective plasmid Maxiprep method for isolation of plasmid DNA from cultured bacterial cells. The GeneKlean® Plasmid Maxiprep Kit is based on alkaline lysis of bacterial cells followed by binding of DNA onto the glass fiber matrix of the spin column in the presence of a high amount of salt. Phenol extraction and ethanol precipitation are not required, and high-quality plasmid DNA is eluted with a small volume of Tris buffer (included

in each kit) or water (pH is between 7.0 and 8.5). Plasmid DNA purified with GeneKlean® Plasmid Maxiprep Kit is suitable for a variety of routine applications including restriction enzyme digestion, Sequencing, library screening, in vitro translation, transfection of robust cells, ligation and transformation. The entire procedure can be completed within 40-50 minutes.

Procedure for Preparation of Maxiprep Plasmid DNA



Bacterial Cells Harvesting

1. Transfer 200 ml bacterial culture to a centrifuge tube.
2. Centrifuge at 6,000 x g for 5 min and discard the supernatant.

Step 1. Resuspend

- Resuspend pelleted bacterial cells in 8 mL Solution 1(RNase A added)

Step 2. Lysis

- Add 8mL of Solution 2 and mix thoroughly by inverting the tube 10 times(Do not vortex) and then stand at room temperature for 2 mins or until the lysate is homologous.

Step 3. Neutralization

- Add 12mL of Solution 3 and mix immediately and thoroughly by inverting the tube 10 times(Do not vortex). Centrifuge at 6,000 x g for 15 min.

Step 4. Binding

- Place a GeneKlean® Plasmid Maxiprep Column in a 50mL centrifuge tube. Apply the supernatant (from Step3) to the GeneKlean® Plasmid Maxiprep Column by decanting or pipetting.
- Centrifuge at 6,000 x g for 3 min. Discard the flow-through and place the GeneKlean® Plasmid Maxiprep Column back into the same 50mL centrifuge tube. Transfer the remaining supernantant to the same GeneKlean® Plasmid Maxiprep Column.
- Centrifuge at 6,000 x g for 3 min. Discard the flow-through and place the GeneKlean® Plasmid Maxiprep Column back into the same 50mL centrifuge tube.

Step 5. Washing

- Add 10mL of Wash Buffer 1 into the GeneKlean® Plasmid Maxiprep Column. Centrifuge at 6,000 x g for 3 min. Discard the flow-through and place the GeneKlean® Plasmid Maxiprep Column back into the same 50mL centrifuge tube.
- Add 12mL of Wash Buffer 2(Ethanol Added) into the GeneKlean® Plasmid Maxiprep Column. Centrifuge at 6,000 x g for 3 min. Discard the flow-through and place the GeneKlean® Plasmid Maxiprep Column back into the same 50mL centrifuge tube.
- Centrifuge at 6,000 x g for 3 min to remove residual Wash Buffer 2.

Step 6. Elution

- Place the GeneKlean® Plasmid Maxiprep Column in a new 50mL centrifuge tube.
- Add 2 mL of Elution Buffer or ddH2O(pH7-8.5) to the center of each column, let stand for 2 min, and Centrifuge at 6,000 x g for 3 min.

Troubleshooting

Problems	Possible Causes	Suggestions
Presence of RNA	RNA Contamination	Ensure RNaseA has added to Solution 1.
Plasmid bands was smeared on agarose gel	Plasmid DNA degradation	Keep plasmid preparations on ice or frozen in order to avoid plasmid DNA degradation
Presence of genomic DNA	Genomic DNA contamination	Do not overgrow bacterial cultures. Do not incubate more than 5 min after adding the Solution 1.
Low yields of DNA	Low plasmid copy number	Increase the culture volume. Change the culture medium.
	Ethanol not Added	Add ethanol to the Wash Buffer 2 before use.
	Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use new glass- and plastic-wares, and wear gloves.
	Column overloaded	Decrease the loading volume or lower the culture density.
	SDS in Solution 2 precipitated	The SDS in Solution 2 may precipitate with storage. If this happens, incubate the Solution 2 at 30~40°C for 5 min and mix well.
	Incorrect elution conditions	Ensure that Elution BUffer is added into the center of the Column.

	Plasmid lost in the host E. coli	Prepare and use fresh culture.
Inhibition of downstream enzymatic reactions	TE buffer used for DNA elution.	Use ethanol to precipitate the DNA, or repurify the DNA fragments and elute with nuclease-free water.
	Presence of residual ethanol in plasmid.	Following the Wash Step, dry the Column with an additional centrifugation step at 6,000 x g for 5 min
DNA passed through in the flow-through or wash fraction	Column overloaded	Check the culture volume. If overgrown, add additional reaction buffer. Check the loading volume.
	Inappropriate salt or pH conditions in buffers	Ensure that any buffer prepared in the laboratory was prepared according to instructions.
Plasmid DNA floats out of wells while running in agarose gel	Incomplete removal of the ethanol	Make sure that no residual ethanol remains in the membrane before eluting the plasmid DNA. Re-centrifuge or vacuum again if necessary.