2180025-2/23

GenCatch™ Plasmid DNA Maxiprep Kit

User's Guide for

Plasmid DNA Purification

For Research Use Only

www.epochlifescience.com

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Quick Start Procedure

This Quick Start Procedure is for Experienced Users Only.
First time users are strongly recommended to read through the detailed instruction protocol in section 4.

Before you start:

1. Add the provided RNase A solution to Buffer VP1, mix and strore at 4 °C.
2. Sit VP3 Buffer on ice before use.

	Cell Pellet
Resuspend in:	**10 ml VP1**
Lyse cell in:	**10 ml VP2 Leave at RT for 5 min.**
Neutralize with:	**10 ml cold VP3 Buffer**
Centrifuge & Equilibrate Column with:	**5 ml 98-100% Ethanol & 10 ml VPN**
Load Super to column:	**Flow by Gravity**
Wash with:	**30 ml VPN**
Elute DNA in:	**10 ml VPE**
Precipitate with:	**7 ml isopropanol**
Wash with:	**10 ml 70 % ethanol**
Air-dry and dissolve in:	**250 µl TE**
	Down Stream Applications

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2 Overview

GenCatch™ plus Plasmid DNA Maxiprep System allows the isolation of ultra-pure plasmid DNA from up to 500 ml culture. DNA binding capacity of GenCatch™ anion-exchange resin is well above 500 µg. An average yield of 500 to 1000 µg of plasmid DNA can be expected. Purified plasmid DNA is suited for use in DNA vaccination, transfection, mutagenesis, in vitro transcription/replication, DNA repair and DNA-binding assays.

Plasmid	Culture Volume	Yield
High copy number	100-150 ml	300 -1000 µg
Low copy number	350-500 ml	300 - 800 µg

Preparation time: 2-2.5 hours.

Downstream Applications:

Plasmid DNA prepared using *GenCatch™ plus Plasmid DNA Maxiprep Kit* is suitable for a variety of molecular manipulations:

- Microinjection
- DNA Vaccine
- Transfection/Transformation
- In vitro transcription/replication
- Restriction enzyme digestion
- Ligation
- Radioactive and Fluorescent sequencing
- PCR
- Library screening or Large-scale screening

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Product Contents

GenCatch™ Plus Plasmid Maxiprep Kit contains sufficient reagents for 10 (Cat. No. 2180010) and 25 (Cat. No. 2180025) plasmid maxipreps, respectively.

	2180010	2180025
VP1 Buffer	120ml	265 ml
VP2 Buffer	120ml	265 ml
VP3 Buffer	120ml	265 ml
VPN Buffer	225ml x2	265 mlx4
VPE Buffer	120ml	265 ml
RNase A (20 mg/ml)	0.60 ml	1.325 ml
GenCatch™ Anion-exchange Column	10	25
GenCatch™ Mini Column	20	50
Protocol	1	1

Add RNase A to VP1 buffer before use and store at 4°C for long term storage.

All components are guaranteed for 48 months from the date of purchase, when stored under specified conditions and used as described in this manual.

Precipitate may form in Buffer VP2 due to cool laboratory temperature, factory storage conditions, or cold temperatures from ambient winter shipping conditions. This will not affect the performance of the product. If a precipitate is observed, warm the bottles to 37°C until it is dissolved. Do not shake VP2 vigorously; SDS present will lead to serious foaming.

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4 Protocol

First time users are strongly recommended to read through this detailed protocol instruction.
For technical support visit: support.epochbiolabs.com

Before you start:

1. Add the provided RNase A solution to Buffer VP1, mix and strore at 4 °C.
2. Sit VP3 Buffer on ice before use.

1. Grow plasmid-containing bacterial cultures in 100-250 ml (high-copy-number plasmids) or 350-500 ml (low-copy-number plasmids) of LB medium with appropriate antibiotic(s) overnight (12-18 hours) with vigorous shaking at 37°C.

2. Harvest the cells by centrifugation at 6,000 x g for 15 minutes.

3. Resuspend the cell pellet in 10 ml of VP1 Buffer.
   Completely resuspend the bacterial cells before adding VP2 Buffer.

4. Add 10 ml of VP2 Buffer, mix gently by inverting the tube 4-6 times and incubate at room temperature for 5 minutes.
   Do not vortex, vortexing will shear genomic DNA. The lysate should be clear and viscous.

5. Add 10 ml of ice-cold VP3 Buffer, gently mix the solution as in step 4.
   After adding VP3 Buffer, white precipitate should be formed.

6. Centrifuge at 20,000 x g for 15 minutes at 4°C.
   20,000 x g corresponds to 12,000 and 13,000 rpm in Beckman JA-17 and Sorvall SS-34 rotors, respectively.

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7. Equilibrate the GenCatchTM Maxi Column with 20 ml of VPN Buffer. Allow the column to empty by gravity flow and discard the filtrate.

8. Apply the supernatant from step 6 to the Maxi Column and allow it to flow through by gravity flow and discard the filtrate.

9. Wash the column once with 30 ml of VPN Buffer by gravity flow and discard the filtrate.

10. Apply 10 ml of VPE Buffer to elute DNA by gravity flow.

11. Precipitate DNA by adding 7.5 ml (0.75 volumes) of room temperature isopropanol to the eluate. Mix and centrifuge at 15,000 x g for 30 minutes at 4°C. Carefully remove the supernatant.

12. Wash the DNA pellet with 5 ml of room temperature 70 % ethanol and centrifuge at 15,000 x g for 10 minutes. Carefully remove the supernatant.

13. Air-dry the DNA pellet for 10 minutes and dissolve the DNA in 300 µl or a suitable volume of TE or ddH2O.

14. Some insoluble material may co-elute with DNA from the column at step 10. To eliminate the insoluble material, load the dissolved DNA solution into a Spin Column (sitting in a 1.5 ml tube) and spin at full speed in a microcentrifuge for 20 seconds.

15. Collect the eluted DNA in the 1.5 ml tube and store at –20°C.

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