2170050-1/09

GenCatch™ Plasmid DNA Midiprep Kit

User's Guide for

Plasmid DNA Purification

For Research Use Only

www.epochlifescience.com

1

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 store at 4 °C.
2. Sit VP3 Buffer on ice before use.

	Cell Pellet
Resuspend in:	**4ml VP1**
Lyse cell in:	**4 ml VP2 Leave at RT for 5 min.**
Neutralize with:	**4 ml VP3 Buffer**
Centrifuge & Equilibrate Column with:	**3 ml 98-100% ethanol and 5 ml VPN**
Load Super to column:	**Flow by Gravity**
Wash with:	**15 ml VPN**
Elute DNA in:	**5 ml VPE**
Precipitate with: And centrifuge	**3.5 ml isopropanol**
Wash with: And centrifuge	**5 ml 70 % ethanol**
Air-dry and dissolve in:	**100 µl TE**
	Down Stream Applications

Epoch Life Science, INC.
13310 S. Gessner Rd.
Missouri city, TX 77489
1-866-395-1362
www.epochlifescience.com

Table of Contents

Epoch Life Science, INC.
13310 S. Gessner Rd.
Missouri city, TX 77489
1-866-395-1362
www.epochlifescience.com

2 Overview

GenCatch™ Plasmid DNA Midiprep System allows the isolation of ultra-pure plasmid DNA from up to 150 ml culture. An average yield of 100 to 150 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	25-50 ml	~100 µg
Low copy number	100-150 ml	~100 µg

Preparation time: 2 hours.

Downstream Applications:

Plasmid DNA prepared using GenCatch™ Plasmid DNA Midi prep 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

Epoch Life Science, INC.
13310 S. Gessner Rd.
Missouri city, TX 77489
1-866-395-1362
www.epochlifescience.com

3 Product Contents

GenCatch™ Plasmid Midi prep Kit contains sufficient reagents for 25 (Cat. No. 2170025) and 50 (Cat. No. 2170050) plasmid midi preps, respectively.

	2170025	2170050
VP1 Buffer	120 ml	265 ml
VP2 Buffer	120 ml	265 ml
VP3 Buffer	120 ml	265 ml
VPN Buffer	265 ml x 2	265ml x4
VPE Buffer	130 ml	265 ml
RNase A 20 mg/ml	0.6 ml	1.325 ml
GenCatch™ Anion-exchange Column	25	50
GenCatch™ Mini Column	50	100
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.

Epoch Life Science, INC.
13310 S. Gessner Rd.
Missouri city, TX 77489
1-866-395-1362
www.epochlifescience.com

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

Grow plasmid-containing bacterial cultures in 25-50 ml (high-copy-number plasmids) or 100-150 ml (low-copy-number plasmids) of LB medium with appropriate antibiotic(s) overnight (12-18 hours) with vigorous shaking at 37°C.

1. Harvest the cells by centrifugation at 6,000 x g for 15 minutes at 4°C.

2. Equilibrate the GenCatch™ Midi Column with 3 ml 98%-100% ethanol. Allow the column to empty by gravity flow and discard the filtrate.

3. Equilibrate the GenCatch™ Midi Column with 5 ml of VPN Buffer. Allow the column to empty by gravity flow and discard the filtrate.

4. Resuspend the cell pellet in 4 ml of VP1 Buffer. *Completely resuspend the bacterial cells before adding VP2 Buffer.*

5. Add 4 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.*

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

Epoch Life Science, INC.
13310 S. Gessner Rd.
Missouri city, TX 77489
1-866-395-1362
www.epochlifescience.com

7. 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.*

8. Apply the supernatant from step 7 to the Midi Column and allow it to flow through by gravity flow and discard the filtrate.

9. Wash the column once with 15 ml of VPN Buffer by gravity flow and discard the filtrate.

10. Apply 5 ml of VPE Buffer to elute DNA by gravity flow.

11. Precipitate DNA by adding 3.5 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 100 µl or a suitable volume of TE or ddH2O.

14. Some insoluble material may co-elute with DNA from the column at step 9. 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. **Note:** the spin column included does not bind DNA. Please don't mix it with our mini spin columns for DNA and RNA purification.

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

Epoch Life Science, INC.
13310 S. Gessner Rd.
Missouri city, TX 77489
1-866-395-1362
www.epochlifescience.com