2160250-2/23

GenCatch™ Plasmid DNA Miniprep 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 **MX1**, mix and store at 4 °C.
2. Add **40 ml** (50 preps) or **180 ml** (250 preps) 98-100% ethanol to Buffer **WS** before use.
3. Add **24 ml** (50 preps) or **120 ml** (250 preps) 98-100% ethanol to **WN** Buffer before use.

	Cell Pellet
Resuspend in:	**200 µl MX1**
Lyse cell in:	**250 µl MX2** Leave at RT for 5 min.
Neutralize with:	**350 µl MX3**
Bind to Column:	**Load and Spin**
Wash with:	**500 µl WN**
Wash with:	**700 µl WS**
Elute DNA in:	**50 µl EB**
	Down Stream Applications

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

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2 Overview

GenCatch™ plus Plasmid DNA Miniprep Kit provides a simple, fast and cost-effective method to purify plasmid DNA without phenol/chloroform extraction. It is based on binding of DNA to silica-based membranes in chaotropic salts. DNA binding capacity of GenCatch™ plus mini column is up to 40 μg. An average yield of 1 to 40 μg of plasmid DNA can be expected from 1 to 5 ml overnight bacterial culture.

Plasmid	Culture Volume	Yield
High copy number	1.5 ml	2-8 μg
	5 ml	20-40 μg
Low copy number	1.5 ml	1-3 μg
	5 ml	5-10 μg

Preparation time: 20-30 minutes.

Downstream Applications:

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

- Radioactive and Fluorescent sequencing
- Restriction enzyme digestion
- Transformation
- Ligation
- PCR
- RAPD, RFLP
- Library screening or Large-scale screening

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Product Contents

GenCatch™ plus Plasmid Miniprep Kit contains sufficient reagents for 50 (Cat. No. 2160050) and 250 (Cat. No. 2160250) plasmid minipreps, respectively.

Catalog Number	2160050	2160250
MX1 Buffer	12 ml	60 ml
MX2 Buffer	15 ml	75 ml
MX3 Buffer	20 ml	100 ml
WN Buffer	6 ml	30 ml
WS Buffer	10 ml	45 ml
Elution Buffer	5 ml	25 ml
RNase A 20 µg/µl	42 µl	210 µl
GenCatch™ plus Column	50 pieces	250 pieces
Collection Tube	50 pieces	250 pieces
Protocol	1	1

Add RNase A to MX1 buffer before use and store at 4°C for long term storage.

Add 40 ml or 180 ml 98-100% ethanol to WS buffer bottle when first opened.

Add 24 ml or 120 ml 98-100% ethanol to WN buffer bottle when first opened.

All components are guaranteed for 24 months from the date of purchase, when stored under specified conditions and used as described in this manual. *GenCatch™ plus* columns should be kept sealed in the zip lock bag provided during storage and away from any heating source.

Precipitate may form in Buffer MX2 and MX3 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 up to 55°C until it is dissolved. Do not shake MX2 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:

Add the provided **RNase A** solution to Buffer **MX1**, mix and store at 4°C.
Add 98-100% ethanol to Buffer **WN** and **WS** before use (see bottle label for volume)

I. Using a Centrifuge:

1. Grow 1 to 5 ml plasmid-containing bacterial cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) with vigorous agitation.

2. Pellet the cells by centrifuging for 1 - 2 minutes. Decant the supernatant and remove all medium residue by pipet.

3. Completely resuspend the cell pellet in 200 µl of MX1 Buffer by vortexing or scratching the bottom of the tube against a tube rack.
   No cell clumps should be visible after resuspension of the pellet.

4. Add 250 µl MX2 Buffer and gently mix (invert the tube 4-6 times) to lyse the cells. Incubate at room temperature for 1 - 5 minutes.
   Do not vortex, vortexing will lead to genomic DNA contamination in the plasmid prep.

5. Add 350 µl MX3 Buffer to neutralize the lysate, and gently mix the solution immediately.
   The white precipitate should be formed.

6. Centrifuge for 5-10 minutes, meanwhile place *GenCatch™ plus* Columns onto a Collection Tube.

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7. Transfer the supernatant carefully to the column.

8. Centrifuge 30-60 seconds at 5000 RPM. Discard the flow-through.

9. Wash the column once with 500 µl WN Buffer by centrifuging for 30-60seconds at 7000x g (9000 RPM). Discard the flow-through.

10. Wash the column once with 700 µl WS Buffer by centrifuging for 30 seconds at 7000x g (9000RPM). Discard the flow-through.

11. Centrifuge the column at 10,000x g (13000 RPM) for another 2 minutes to remove residual ethanol.

    It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

12. Place the column onto a new 1.5-ml centrifuge tube. Add 50 µl of Elution Buffer (provided) onto the center of the membrane.

    For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

13. Stand the column for 2 minutes at room temperature (optional, for best recovery of DNA) and centrifuge for 30 seconds at the same speed as used in step 11 and elute DNA.

14. Store plasmid DNA at 4 °C or –20 °C.

II. Using a Vacuum Manifold:

The following protocol uses a vacuum manifold (not provided in this kit).

Follow step 1 through step 5 in previous protocol.

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6. Centrifuge for 5 - 10 minutes, meanwhile insert the tip of a *GenCatch™ plus* Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man*).

7. Transfer the supernatant carefully into the column.

8. Apply vacuum to draw all the liquid into the manifold.

9. Wash the column once with 500 µl WN Buffer by re-applying vacuum to draw all the liquid.

10. Wash the column once with 700 µl WS Buffer by re-applying vacuum to draw all the liquid.

    It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

Place the column onto a Collection Tube and proceed to step 11 through step14 in protocol I.

* Vac-man is a trademark of Promega Corporation.

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5 Troubleshooting Guide

The following guide addresses some of the most common problems. A database of user raised questions and answers are being build at **support.epochbiolabs.com**.

Problem	Possible Reasons	Solution
Poor bacterial growth	Inoculate bacterial cells from a plate or a culture stock stored for long time	Always inoculate bacterial cells from a freshly streaked plate and grow with required antibiotic(s)
	Incubation with inadequate shaking	Grow cells with vigorous shaking (e.g. 250 rpm). Adjust a suitable shaking speed according to the angular magnitude of an orbital shaker platform.
Poor cell lysis	Use too many bacterial cells harvested from a large culture or an over-grown culture	Up to 5 ml culture for high-copy plasmid Up to 10 ml culture for low-copy plasmid. When the culture is more than 5 ml, use double amount of MX1 MX2, and MX3.
	Cell pellet is not well resuspended	Do not add MX2 buffer until cells are completely resuspended by vortexing.
Low yield of plasmid DNA	Not enough bacterial cells	Ensure that bacteria have grown well after overnight incubation with vigorous shaking.
	Overgrowth of bacteria	Do not incubate for more than 16 hours.
	Plasmid does not propagate	Always inoculate bacterial cells from a freshly streaked plate and grow with required antibiotic(s)
	Inefficient or incomplete DNA elution	Efficient and complete DNA elution only takes place when elution solution is within pH 7-8.5 and is full contact with the membrane. Make sure that no less than 30 µl of solution is dispensed onto the membrane.

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RNA contamination	RNase A is absent from MX1 Reduced RNase A activity due to improper or prolonged storage.	Add the supplied RNase to MX1 Add RNase A to MX1 to a final concentration of 50 ug/ml and store at 4°C
Genomic DNA contamination	Prolonged incubation with MX2 or vigorous shaking in step 4	Do not vortex, mix gently, and keep incubation within 5 minutes.
Low yield of plasmid larger than 10 Kb		Use elution solution preheated to 70°C

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