Plasmid DNA miniprep protocol for EZ-10 Spin Column Plasmid DNA minipreps kit (BS413), 50 preps Version 3

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Abstract

This protocol is for the extraction and purification of up to 10ug of plasmid DNA. It can be used for the purification of plasmid DNA ranging from 40 bp to 40kbp.

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Guidelines

Introduction

The EZ-10 Spin Column Kits provide a simple and efficient method for purification of plasmid DNA, extraction of DNA from agarose gels, and purification of DNA from enzymatic reactions such as PCR or restriction enzyme digestions.

The DNA is selectively adsorbed in silica gel-based EZ-10 column and other components are washed away. The DNA is then eluted off the column and can be used for any downstream applications.

The purification method used in these protocols does not require use of phenol, chloroform, or CsCl. The DNA is purified without an additional step of ethanol precipitation.

Limitations of Use

These kits are designed for research use only. The purified plasmid DNA should not be used for live animal transfections. It is also not to be used for human diagnostic or drug production purposes.

Features

- Simple, Fast and Efficient
- Preparation of high quality DNA which can be used in any downstream applications such as sequencing, PCR, transformation or restriction digestions
- HighYield and Reproducible
- High Capacity -Up to 10µg of DNA per column

Storage

EZ-10 Spin Column Kits should be stored dry at room temperature (15 °C-25 °C). Kits can be storedfor up to 24 months without showing any reduction in performance and quality. RNase A stock solution can be stored for 2 years at 4 °C. After addition of RNase A, Solution I is stable for 6 months at 4 °C.

Quality Control

Each lot of EZ-10 Spin Column kit is tested against predetermined specifications to ensure consistent product quality.

Principle:

This kit provides a simple and efficient method formini plasmid DNA purification. The plasmid DNA is selectively adsorbed in silica gel-based EZ-10 column and other impurities such as proteins, salts,nucleotides, oligos (<40-mer) are washed away. In order to maximize the recovery yield of plasmid DNA, a color indicator-VisualLyse is added to the buffer which prevents insufficient or over-lysis during lysis and neutralization step. The plasmid DNA is then eluted off the column and can be used for any downstream application.

Before start

1) Add RNAse A solution to the bottle containing Solution 1 and mix well. Once RNAse A solution is added to solution 1, the resulting solution is stable for 6 months at 4°C. If being used infrequenly the solution can be stored at -20 °C for longer periods as long as freeze thaw cycles are minimized.

2) If solution 2 contains a precipitate, dissolve the precipitate before use by gently warming the solution at 37 °C.

3) Before using the wash solution, add 80 mL of 96-100% Ethanol to the 20 mL wash solution. Anhydrous Ethanol from Bio Basic Inc. (D0193) is suitable for use with this protocol.

Materials

EZ-10 Spin Column Plasmid DNA Miniprep Kit BS413.SIZE.50preps by Bio Basic Inc.

1.5ml Tube, Natural, 1000/Bag BT620-N.SIZE.1PK by Bio Basic Inc.

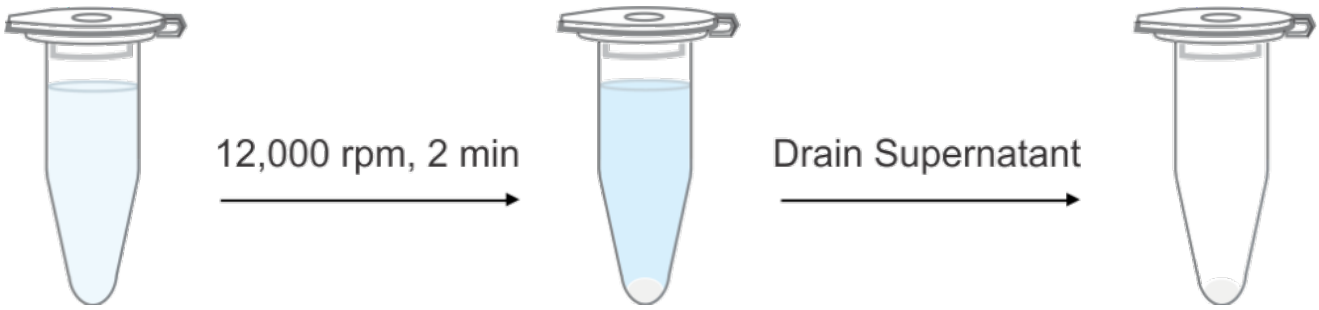
Ethanol, anhydrous (85%) D0193.SIZE.500ml by Bio Basic Inc.

Protocol

Step 1.

Add 1.5 mL of overnight culture to a microcentrifuge tube and centrifuge at 12,000rpm for 2 minutes. Drain the clarified supernatant completely leaving only the cell pellet.

Optional: Depending on ease of plasmid replication add a further 1.5 mL of overnight culture to the microcentrifuge tube containing the previous pellet and repeat until a maximum of 5 mL of overnight culture has been added.



DURATION

00:02:00

Step 2.

Add 100uL of **Solution 1** to the pellet, mix well, ensuring that no clumps are present and let sit for 1 min

DURATION

00:01:00

Step 3.

Add 1µL of VisualLyse to the solution from step 2 (optional)

Step 4.

Add 200µl of **Solution II** to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute. To prevent contamination from genomic DNA, do not vortex.

DURATION
00:01:00

NOTES
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If VisualLyse has been added, the solution will turn blue after addition of Solution II. A homogenously blue suspension should then be observed. If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.

Step 5.

Add 350µl of **Solution III**, and mix gently. Incubate at room temperature for 1 minute. A fluffy white material forms and lysate should become less viscous. If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless.

DURATION
00:01:00

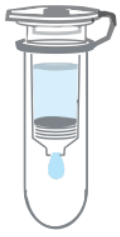
Step 6.

Centrifuge at 12,000rpm for 5 minutes

DURATION
00:05:00

Step 7.

Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes



DURATION
00:02:00

Step 8.

Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes

DURATION
00:02:00

Step 9.

Repeat wash procedure in step 8

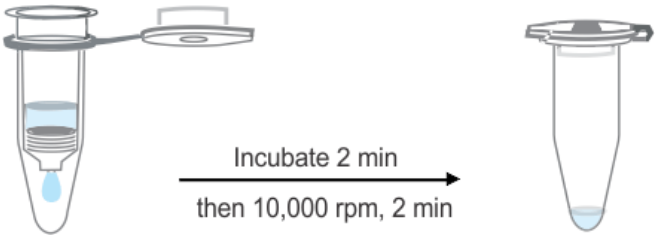
Step 10.

Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional minute to remove any residual Wash Solution

DURATION
00:01:00

Step 11.

Transfer the column to a clean 1.5ml microfuge tube (BT620-NS). Add 50µl of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes.



REAGENTS
1.5ml Tube, Natural, Sterile, 1000/Bag BT620-NS.SIZE.1PK by Bio Basic Inc.

DURATION
00:02:00

NOTES
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Note: It is extremely important to add the Elution Buffer into the center part of the column.

Incubating the column with the Elution Buffer at higher
temperature (37ºC to 50ºC) may slightly increase the yield especially for large (>10,000bp) DNA Plasmids. Prewarming the Elution Buffer at 55ºC to 80ºC may also slightly increase elution efficiency.

Step 12.

Centrifuge at 10,000 rpm for 2 minutes

DURATION
00:02:00

Step 13.

Store purified DNA at -20ºC