Plasmid DNA Miniprep Kit

EZ-Spin Column Plasmid DNA Miniprep Kit provides a simple and efficient

method for purification of plasmid DNA.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

EZ-Spin Column Plasmid DNA Miniprep Kit

Catalog Number: MB1005

Handling and Storage

Storage and handling upon receiving: Store the kit dry at room temperature 15°C to 25°C for up to 24 months. 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.

Introduction

The EZ-Spin Column Plasmid DNA Miniprep Kit provides a very fast and simple plasmid miniprep method for routine molecular biology laboratory applications. This innovative technology has dramatically transformed traditional lysis-based preps by significantly reducing the number of steps associated with conventional processes.

DNA is selectively adsorbed in silica gel-based EZ-10 column and other components/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 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. The yield can be Up to 10μg of DNA per column.

Kit Components

3 sizes of MB1005 are available. The kit components listed below:

Components	**MB1005-50**	**MB1005-100**	**MB1005-250**
RNase A Solution (10mg/ml)	120μl	240μl	600μl
Solution I	6ml	12ml	30ml
Solution II	12ml	24ml	2x30ml
Solution III	25ml	2x25ml	5x25ml
VisualLyse (color indicator for lysis)	60μl	120μl	300μl
Wash Solution	20ml	2x20ml	2x40ml
Elution Buffer	5ml	10ml	25ml
EZ-10 Column	50	100	250
Datasheet	1	1	1

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs).

Liquid bacterial growth medium supplemented with the appropriate antibiotic

Incubator and basic microbiology equipment for bacterial culture Ice

Microcentrifuge Vortexer*

Isopropanol (95–100%) 2 ml microcentrifuge tubes Pipets and pipet tips

Note Prior To Starting Experiment

1. Before use, add the RNase A Solution to the bottle containing Solution I and mix well. Solution I with RNase A should be stored at 4ºC for frequent use and at -20ºC for infrequent use.
2. Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37ºC.
3. Before use, add 80ml of 96-100% of ethanol to 20ml Wash Solution for MB1005-50; add 160ml of 96-100% ethanol to 40ml Wash Solution for MB1005-100; add 320ml of 96-100% ethanol to 80ml Wash Solution for MB1005-250. For other volumes of wash solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol: volume of Wash Solution = 4:1).
4. Elution Buffer is 2.0 mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water may be substituted, the resulting yields may be up to 20% lower.

Protocol For Plasmid DNA Purification

1. Add 1.5 - 5mL overnight culture in the tube and centrifuge at 12,000rpm for 2 minutes. Drain the liquid completely. For low copy number plasmid, see the protocol on the following page.
2. Add 100µl of Solution I to the pellet, mix well, and keep for 1 minute.
3. Add 1µl of VisualLyse to the mixture above. Note: addition of VisualLyse is an optional step.
4. Add 200µl of Solution II to the mixture, 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. If VisualLyse has been added, the solution will turn blue after addition of Solution II. A homogenously blue suspension should also be observed. If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.
5. Add 350µl of Solution III, 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.
6. Centrifuge at 12,000rpm for 5 minutes.
7. Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes.
8. Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
9. Repeat wash procedure in step 8.
10. Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional minute to remove any residual Wash Solution. Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional minute to remove any residual Wash Solution.
11. Transfer the column to a clean 1.5ml microfuge tube. Add 50µl of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes.
12. Store purified DNA at -20ºC.

Note: It is 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.

Troubleshooting Guide

1. Low Yield: There are a number of variables that can cause low yield:
   a. Each of the steps has to be strictly followed.
   b. Make sure there is no precipitant in Solution I, II or III. If precipitant is present in the buffer, warm up the solution to 37°C and shake well.
   c. Low culture density. Make sure that the temperature in the incubator is stable and shaking speed provides sufficient aeration of the culture.
   d. Very high cell density, therefore incomplete cell lysis. Double the volume of Solution I, II and III.
2. Contamination of chromosomal DNA:
   a. Do not vortex the sample after adding solution II and III. Vigorous shaking will cause shearing of chromosomal DNA. Smaller pieces of chromosomal DNA will be captured on silica gel and carried over with purified plasmid DNA.
3. RNA contamination
   a. RNase activity is weakened or lost. Add addition RNase A to Solution I and store at 4°C.
4. OD 260nm/280nm ratio is outside 1.6-1.8 range:
   a. If the ratio of OD260nm/280nm is greater than 1.8, there may be traces of ethanol present.
   b. If the ratio of OD260nm/280nm is smaller than 1.6, there is chance of protein and salt contamination. Make sure the sample is mixed well after Solution III is added and after spinning down. In addition, repeat one more wash step before elution step to remove extra salt completely.
5. For optimal results in downstream DNA sequencing, an additional washing step is recommended.

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