【Interpretation of test results】

1. No plasmid DNA was extracted.

If there is no plasmid DNA in the Elution Buffer after elution, please check whether absolute ethanol was added to the Wash Buffer according to the volume indicated on the bottle label.

2. Plasmid extraction rate is low.

1) Please confirm the cultured bacteria first, and exclude the contamination of bacteria and the loss of plasmids during the culture process.

2) After adding the Resuspension Buffer, the bacterial precipitate should be fully suspended and dispersed.

3. Problems with electrophoresis results

1) If there is the genomic bands problem: After adding Lysis Buffer and Neutralization Buffer, invert the centrifuge tube gently to avoid shearing of genomic DNA fragments.

2) If there is an RNA contamination problem: please add RNase A to the resuspension to a final concentration of 100μg/mL.

【Limitations of the test method】

The sample volume should not exceed 5mL high-copy plasmid bacteria.

【Product Performance Index】

Extraction product detection (OD260-OD320)/(OD280-OD320) ratio: 1.8-2.0.

【Notes】

The following procedures are suitable for use with the Bioer NPA-32P nucleic acid purification instrument. If other nucleic acid purification instruments are used, according to different instruments need to adjust the operating procedures.

【Company Information】

Manufacturer: Hangzhou Bioer Technology Co.,Ltd

Address: No.1192 Bin'An Rd, Binjiang District, Hangzhou, Zhejiang Province, China

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Aftersales Service Provider: Hangzhou Bioer Technology Co.,Ltd

MagaBio plasmid DNA Purification Kit

【Product Name】 MagaBio plasmid DNA Purification Kit

【Packing Size】 32 Tests/box; 50 Tests/box; 100 Tests/box

【Usage】 Used to extract plasmid DNA from 1～5 mL overnight cultures of *Escherichia coli.*

【Principle and Advantage】

This kit uses a special buffer system and magnetic beads, which can separate and purify 1～20 μg of high-quality plasmid DNA from 1～5mL bacterial solution.

Under the action of the Binding Buffer, the released DNA is specifically bound to the magnetic beads, and the DNA-bound magnetic beads are captured by the magnetic material, and the contaminants are removed through two washing processes. DNA is eluted and collected from the magnetic beads under the action of the Elution Buffer.

Plasmid DNA extracted with this kit can be used for various molecular biology experiments, such as enzyme digestion, sequencing, library screening, ligation and transformation.

【Kit Components】

Cat#	**BSC41S1E**	**BSC41S1**	**BSC41M1**
Components	**32T**	**50T**	**100T**
RNase A	32μL	50μL	100μL
Resuspension Buffer	8mL	12.5mL	25mL
Lysis Buffer	8mL	12.5mL	25mL
Neutralization Buffer	4.8mL	7.5mL	15mL
Binding Buffer	96 well pre-packed plate 2 pieces	50mL	100mL
Wash Buffer		12mL×2 （Add 28mL ethanol before use）	24mL×2 （Add 56mL ethanol before use）
Elution Buffer		10mL	20mL
Maganetic beads		0.6mL	1.2mL
Handbook	1	1	1

【Reagents to be prepared by the user】

Buy BSC41S1 and BSC41M1, please prepare the absolute ethanol (analytical grade) by yourself.

【Storage and Validity period】

1) The kit should be stored at 2～8℃. If all reagents are stored as above, they are stable for 18 months.

2)The kit can be transported at room temperature (2～25℃).

【Applicable instrument】

Magnetic rack or Bioer NPA-32P purification instrument; Water bath or dry bath; Vortex mixer; centrifuge.

【Sample Requirements】

1～5mL of overnight culture of *Escherichia coli*.

【Procedure】

1. Sample preparation and pre-processing.

1) Add 1～1.5mL overnight cultured bacterial solution to a 1.5mL centrifuge tube.

2) Centrifuge at 10,000rpm (8,000～10,000×g) for 30 seconds and discard the supernatant.

Note: If necessary, repeat steps 1) and 2) multiple times to collect more bacterial cells. But do not excessive (not more than 5ml), so as not to affect the quality of the extracted plasmid.

3) When using for the first time, please transfer all the RNase A solution into the Resuspension Buffer, and store the Resuspension Buffer at 2～8℃.

4) Add 250 µL Resuspension Buffer to resuspend the bacterial cells.

Note: There should be no bacterial clumps after resuspension.

5) Add 250 µL Lysis Buffer and invert gently 4～6 times.

Note: Do not shake vigorously to avoid fragmentation of genomic DNA. Be careful not to let the reaction time exceed 5 minutes.

6) Add 150 µL Neutralization Buffer and immediately invert gently the centrifuge tube 4～6 times.

Note: The solution should appear flocculent, but there should be no local precipitation.

7) Centrifuge at 13,000 rpm (>14,000×g) for 10 minutes.

Note: If the centrifuge speed is not enough, the centrifugation time can be extended until a dense white precipitate is formed.

2. Magnetic Bead Binding

1) Transfer 600µl supernatant to a new 2.0mL centrifuge tube.

2) Add 1mL Binding Buffer to the supernatant of the centrifuge tube and mix by shaking.

3) Add 12 µL mixed Magnetic Beads, close the tube cap tightly, gently invert the mixed centrifuge tube, and let it stand at room temperature for 10 minutes **(Note: Use a rotary shaker or manually mix every 2～3 min)**.

3. DNA purification

1) Place the centrifuge tube on the magnetic rack for 1 minute to allow the magnetic beads in the tube to be adsorbed, use a pipette to remove the liquid in the tube, and remove the centrifuge tube.

2) Add 650µL Wash Buffer into the centrifuge tube, close the cap tightly, and invert the centrifuge tube several times to ensure that the magnetic beads are completely dispersed. Place the centrifuge tube on the magnetic rack for 1 minute, use a pipette to remove the liquid in the tube, and remove the centrifuge tube.

3) Follow step 2) to wash again.

4) Open the centrifuge tube lid to dry for 5 minutes at room temperature.

5) Add 50～100µL Elution Buffer, gently mix the centrifuge tube and incubate at 70℃ for 5minutes. (**Note: Mix gently every 2 minutes.)**

6) Place the centrifuge tube on the magnetic rack for 1 minute to adsorb the magnetic beads, and transfer the liquid to a new 1.5 mL nuclease-free centrifuge tube, a please store at -20℃.

Appendix: The automation purification, take Bioer NPA-32P as an example

1. Reagent Preparation

A. For BSC41S1 and BSC41M1.

Add 500µL Binding Buffer to the column 1, 2 and 7, 8 of the 2.2mL 96-deep-well plate. Add 650µL Wash Buffer to the column 3, 4 and 9, 10. Add 60µL Elution Buffer to the column 5 and 11. Add 188µL pure water and 12µL MagaBio Reagent to the column 6 and 12 (the magnetic beads should be mixed thoroughly before use).

B. For BSC41S1E.

Put the 96 well pre-packed reagents at room temperature. Shake 96-well plate upside down for three times, and tear off the plastic bag. Centrifuge the pre-packed reagent for a few seconds (or swing by hand a few times) to avoid reagent adhering to the wall of the tubes. Tear off the aluminum foil film of 96-well plate and identify the direction of the plate (magnetic beads in column #6 & #12)

2. Sample preparation and pre-processing.

1) The sample preparation and pre-processing the same as manual extraction (see sample preparation and pre-processing procedure).

2) Transfer 300µL supernatant to the 96 deep well plate columns 1, 2 or 7, 8 of the 96-well plate, respectively. Please avoid cross-contamination.

3) Place 96 deep well plate to the instrument, install the 8-strip tips on the instrument.

3. Run the program according to the following procedures:

Step	Well	Name	Waiting Time （min ： ss）	Mixing Tine （min ： ss）	Magnet Time （min ： ss）	Adorption	Speed	Volume （µL）
1	6	Beads	00 : 00	00 : 15	00 : 30	Strong	F	200
2	1	Binding	00 : 00	05 : 00	01: 00	Strong	F	800
3	2	Binding	00 : 00	05 : 00	01: 00	Strong	F	800
4	3	Wash 1	00 : 00	03 : 00	01: 00	Strong	F	650
5	4	Wash 2	00 : 00	02 : 00	01: 00	Strong	F	650
6	5	Elution	02 : 00	05 : 00	01: 00	Strong	F	100
7	6	Discard	00 : 00	01: 00	00 : 00	Normal	F	200

Heating setting: Elution temperature: 65℃. Elution starts heating at Step 6

4. After the automatic purification is over, transfer the Elution Buffer in columns 5 and 11 to a new 1.5mL nuclease-free centrifuge tube; if not using it immediately, please store at -20℃.