Mag Beads High Adsorption Plasmid Extraction Kit

【Product Name】

Mag Beads High Adsorption Plasmid Extraction Kit

【Package Specifications】

50T/box（Art.No.SP102-50）；100T/box（Art.No.SP102-100）；

300T/box（Art.No.SP102-300）；500T/box（Art.No.SP102-500）

【Intended Use】

This product is suitable for the extraction of plasmid DNA, which can be subsequently used in various molecular biology experiments such as sequencing, enzymatic digestion, ligation, transformation, and library screening.

【Detection Principle】

DNA binds to the surface of silicon-coated Magbeads under high salt conditions. After multiple washes to remove impurities such as proteins, DNA is eluted under low salt conditions, resulting in high-purity DNA.

【Main Components】

Components	SP102-50	SP102-100	SP102-300	SP102-500
Buffer1	16mL	31mL	95mL	155mL
Buffer2	16mL	31mL	95mL	155mL
Buffer3	8mL	16mL	48mL	80mL
Bead Suspension	1.1mL	1.1mL×2	6.5mL	10.5mL
Washing Buffer	8mL（+18.64mL Anhydrous Ethanol）	8mL（+18.64mL Anhydrous Ethanol）	8mL（+18.64mL Anhydrous Ethanol）	8mL（+18.64mL Anhydrous Ethanol）
Eluant	6mL	12mL	35mL	55mL
RNaseA	160μL	310μL	950μL	1.55mL

【Storage Conditions and Shelf Life】

Magnetic bead suspension should be stored at 2-8°C, RNase A should be stored below 4°C, and other reagents should be stored at room temperature. All components have a shelf life of one year. Transportation can be done between 4-37°C for a maximum of 14 days.

【Precautions】

1) Before use, thoroughly mix RNase A into Buffer 1 and store at 2-8°C. If stored for more than 6 months, RNase A should be supplemented to a concentration of 10mg/mL (mixing ratio of Buffer 1 to RNase A is 100:1).

2) Before use, check for salt precipitation in Buffer 2 and Buffer 3. If present, they can be re-dissolved at 37°C.

【User-provided Equipment and Reagents】

Equipment: Vortex mixer, constant temperature shaker, magnetic rack, pipettes, tips, centrifuge, centrifuge tubes, 96 deep-well plates, etc.

Reagents: Lysozyme, 80% ethanol, isopropanol.

【Manual Operation Steps - Centrifuge Tube Operation】

1. Take 2-5mL of bacterial suspension into a suitable centrifuge tube, centrifuge at 12000g for 2 minutes, and discard the supernatant. Fully suspend the bacterial precipitate in 300μL Buffer 1 (confirm if RNase A is added).

2. Add 300μL Buffer 2 and gently invert 5-6 times to fully lyse the bacteria and form a clear solution.

3. Add 150μL Buffer 3, gently invert 6-8 times to form a compact flocculent mass. Centrifuge at 12000g for 10 minutes at room temperature, transfer 600-700μL supernatant to a new centrifuge tube, add 20μL magnetic beads, and 400μL isopropanol. Shake at

room temperature for 5 minutes to combine.

4. Place the centrifuge tube on the magnetic rack and let it stand for 30 seconds until the solution clarifies, then remove the supernatant as much as possible.

5. Add 500μL wash solution, vortex for 30 seconds, resuspend the magnetic beads, then place the centrifuge tube on the magnetic rack for 30 seconds until the solution clarifies, and remove the supernatant.

6. Add 500μL 80% ethanol, vortex for 20 seconds, resuspend the magnetic beads, then place the centrifuge tube on the magnetic rack for 30 seconds until the solution clarifies, remove the supernatant, and remove residual droplets from the tube wall as much as possible.

7. Repeat step 6 once.

8. Ventilate in a fume hood for 5 minutes (ensure ethanol is completely evaporated).

9. Add 50-100μL elution buffer to the magnetic beads, vortex thoroughly, and shake at room temperature for 5 minutes (for better results, shake at 55°C constant temperature for 5 minutes).

Note: Before adding the elution buffer, it can be heated in a water bath to 55°C to facilitate better elution of plasmid DNA.

10. Place the centrifuge tube on the magnetic rack. After magnetic absorption is complete and the solution is clear, aspirate the supernatant and transfer it to a clean centrifuge tube for storage at -20°C.

【Manual Operation Steps - 96-Well Plate Operation】

1. Add 2-5mL of overnight cultured bacterial solution to a 96 deep-well plate, centrifuge at 4000g for 15 minutes, discard the supernatant, and then invert the deep-well plate on absorbent paper for 2 minutes.

2. Add 300μL of Buffer 1 (confirm if RNase A is added), and thoroughly shake on a vortex mixer to fully disperse the bacteria.

Note: If the bacteria are not completely suspended, it may affect the subsequent lysis efficiency, resulting in decreased concentration and purity of plasmid DNA.

3. Add 300μL of Buffer 2 to each well, seal the membrane, invert 5-10 times, and let it stand for 1 minute to fully lyse the bacteria.

Note: Do not vigorously shake to avoid breaking genomic DNA. (This operation should take less than 5 minutes)

4. Add 150μL of Buffer 3 to each well, seal the membrane, invert more than 10 times, and let it stand for 1 minute.

5. Centrifuge the 96 deep-well plate at 4000g for 15 minutes.

Note: If there is still white precipitate in the supernatant after centrifugation, or if the sediment at the bottom of the well is loose, continue centrifugation for 10 minutes.

6. Transfer 600-700μL of supernatant (as much as possible) to a new 96 deep-well plate, then add 20μL thoroughly mixed magnetic bead suspension and 400μL isopropanol. Shake with a shaker for 5 minutes.

7. Place the 96 deep-well plate on the magnetic rack, let it stand for 30 seconds, discard the supernatant when it clears.

8. Remove the 96 deep-well plate from the magnetic rack, add 500μL wash solution using a pipette, shake on a shaker for 30 seconds, then place the 96 deep-well plate on the magnetic rack, let it stand for 30 seconds, and discard the supernatant when it clears.

9. Remove the 96 deep-well plate from the magnetic rack, add 500μL 80% ethanol using a pipette, shake on a shaker for 30 seconds, then place the 96 deep-well plate on the magnetic rack, let it stand for 30 seconds, and discard the supernatant when it clears.

10. Repeat step 9 once.

11. Keep the 96 deep-well plate on the magnetic rack and invert it onto a clean absorbent paper for 2 minutes.

12. Ventilate in a fume hood for 5 minutes (ensure ethanol is completely evaporated).

13. Remove the 96 deep-well plate, add 50-100μL elution buffer to each well, and place the 96 deep-well plate in a 55°C constant temperature shaker for 5 minutes.

14. Place the 96 deep-well plate on the magnetic rack and let it stand for 30 seconds. After the magnetic beads are completely adsorbed to the side wall of the round hole plate, use a pipette to transfer the elution buffer to a new deep-well plate or PCR plate, and store at -20°C for later use.

【Automated Operation Steps for 96-Channel Nucleic Acid Extractor】

1. Sample Preparation:

Add the specified amounts listed in the table to each corresponding well of a 96-well plate, which can simultaneously process 96 samples.

Position	1	2	3	4	5	6
Reagent	Isopropanol（400μL）	Beads（20μL） Sterilization Water（200μL）	Washing Buffer（500μL）	80% Ethanol（500μL）	80% Ethanol（500μL）	Eluant 100μL

2. Sample Processing:

1) Add 2-4mL overnight cultured bacterial liquid into a 48 deep-well plate (if the bacterial liquid is cultured in a 48 deep-well plate, it can be centrifuged directly according to the following steps), centrifuge at 4000g for 15 minutes, discard the supernatant, and then invert the deep-well plate on absorbent paper for 2 minutes.

2) Add 300μL Buffer1 (confirm whether RNase A is added), thoroughly vortex the plate on a shaker to completely disperse the bacterial cells.

Note: Failure to completely suspend bacterial cells will affect the subsequent lysis efficiency, resulting in decreased plasmid DNA concentration and purity.

3) Add 300μL Buffer2 to each well, seal the plate, invert it 6-10 times, and let it stand for 1 minute to allow full cell lysis.

Note: Do not vigorously shake the mixture to avoid interrupting genomic DNA (this operation should be completed in less than 5 minutes).

4) Add 150μL Buffer3 to each well, seal the plate, invert it 6-10 times, and let it stand for 1 minute.

5) Centrifuge the 48 deep-well plate at 4000g for 15 minutes, transfer 600-700μL supernatant to a 96 deep-well plate at position 1.

Note: If there is still white precipitate in the supernatant after centrifugation, or if the precipitate at the bottom of the well is loose, continue centrifugation for 10 minutes, and then transfer the clarified supernatant. Optionally, when transferring the supernatant after centrifugation, it can be first transferred to a 96-well filter plate (previously placed on position 1 of the 96 deep-well plate), centrifuged at 4000g for 2 minutes to remove remaining impurities.

3. Automated Extraction:

Place the prepared 96-well sample plate in sequence into the nucleic acid extractor (QN-AUT-96) or a similar type of nucleic acid extractor, and insert the magnetic rod sleeve; open the operation program of the instrument, call up the corresponding program, click "run" to start the extraction.

4. Nucleic Acid Transfer:

After the automated program is completed, seal and store the eluate or transfer it to a

new sample plate for storage at -20°C.

The parameters for the 96-channel nucleic acid extractor (QP-AUT-96) program are set as follows

Procedure	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
Station	2	1	3	4	5	6	5
Waiting time	00:00:00	00:00:00	00:00:00	00:00:00	00:00:00	00:03:00	00:00:00
Mixed model	2	2	2	2	2	2	2
Mixing time	00:00:30	00:05:00	00:02:00	00:01:00	00:01:00	00:05:00	00:00:30
Suspend	No	No	No	No	No	No	No
Suction time	00:01:00	00:01:00	00:01:00	00:01:00	00:01:00	00:01:00	00:00:00
Volume	200μL	1000 μL	500 μL	500 μL	500 μL	100 μL	500 μL
Temperature	—	—	—	—	—	55℃	—

【Basic Information】

Version Number: 1.1

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