Code.:BR2D102

Endo-free Plasmid Maxi Kit

V02

Product Description

The Endo-free Plasmid Maxi Kit utilizes a unique silica membrane adsorption technology to bind plasmid DNA with high efficiency and specificity. The extracted plasmid is of high purity and low endotoxin content, with a high recovery rate of 80%-90%. The whole experimental procedure of plasmid DNA extraction could be finished within 1 h. Plasmids purified with this kit can be used for various routine procedures, including Restriction Digestion, PCR Sequencing, Ligation, Transformation, Gene Therapy, Cell Microinjection, Gene Silencing, and Cell Transfection experiments.

Components

Component	BR2D102-00 2 T	BR2D102-01 10 T
Buffer BL	10 mL	30 mL
Buffer E1	30 mL	100 mL
Buffer E2	30 mL	100 mL
Buffer E3	30 mL	100 mL
Buffer PW	15 mL	70 mL
Buffer TB	5 mL	30 mL
RNase A（10 mg/mL）	0.35 mL	1.2 mL
Filtration CS1	2	10
Spin Columns CP1	2	10
Collection Tubes 50 mL	4	20

Storage

Store at 15°C-30°C.

Materials

Anhydrous ethanol, isopropanol.

Notes

1. For Research Use Only. Not for use in diagnostic procedures.

2.Before the first use, add all RNase A to Buffer E1 as per the instructions, mix well and store at 2°C-8°C for stable storage .

3.Before use, please check Buffer E2 and Buffer E3 for any crystallization or precipitation. If there is any crystallization or precipitation, they can be restored to clarity in a 37°C water bath for a few minutes.

Note: Buffer E2 and Buffer E3 contain irritating substances, please wear gloves to operate, and tighten the lid immediately after use.

4.Add ethanol to Buffer PW before use. For Biori#BR2D102-01, add 165 mL Anhydrous ethanol; For Biori#BR2D102-00, add 35 mL Anhydrous ethanol.

5. When using the filtration column, pull the plunger out of the column to avoid loosening the filter membrane due to pressure.

6. For low-copy-number plasmid or high-molecular-weight plasmid (>10 kb) , increase the amount of bacterial culture used. Correspondingly increase the volumes of Buffer E1, Buffer E2, and Buffer E3 proportionally. Pre-warm the Buffer TB in a 65°C-70°C water bath. Appropriately extending the adsorption and elution times may improve the yield.

7. Recommended culture volume for high-copy-number plasmid is 100 mL, with a typical yield of 500 μg-1500 μg. For low-copy-number plasmid, use 200 mL culture, with a typical yield of 200 μg-600 μg.

Protocol

1.Equilibration of the Adsorption Column: Add 2.5 mL of Buffer BL to the Spin Column CP1 (placed in a 50 mL Collection Tube) . Centrifuge at 8,000 rpm for 2 min at room temperature. Discard the flow-through and place the Spin Column CP1 back into the Collection Tube.

Note: The adsorption column preconditioned with equilibration buffer should be used as soon as possible.

2.Harvest bacterial cells from 100-200 mL of overnight culture (the volume is based on cell density) by centrifugation at 8,000 rpm for 2-3 min at room temperature in a centrifuge tube (Self-prepared materials) . Carefully remove the supernatant.

Note: When the volume of bacterial culture is large, the bacterial pellet can be collected into a single centrifuge tube via repeated centrifugation. The optimal volume of bacterial culture is sufficient for complete lysis excessive bacterial culture will result in incomplete lysis, thereby reducing plasmid extraction efficiency. If the bacterial biomass is high, simultaneously increase the volume of Buffer E1, Buffer E2, and Buffer E3 by 1-2 mL proportionally.

3. Add 8 mL of Buffer E1 (ensure RNase A has been added) to the tube with the bacterial cell pellet. Resuspend the pellet thoroughly using a pipette or vortex mixer.

Note: Complete resuspension is critical. Unresolved clumps will reduce lysis efficiency, leading to lower yield and purity.

4. Add 8 mL of Buffer E2 to the tube. Immediately mix by gently inverting the tube 6-8 times to lyse the bacterial cells completely. Incubate at room temperature for 5 min.

Note: Mix gently to avoid shearing genomic DNA, which can contaminate the plasmid prep. The solution should become clear and viscous. If the solution won't turn clear, please reduce the amount of bacterial cells.

5. Add 8 mL of Buffer E3 to the tube. Immediately mix by gently inverting 6-8 times until white flocculent precipitates form uniformly. Incubate at room temperature for 10 min. Centrifuge at 8,000 rpm for 10 min at room temperature to pellet the precipitate (centrifugation time can be extended to 20-30 min if needed) . Carefully pour all the supernatant into a Filtration CS1, avoiding transfer of large precipitate particles. Slowly push the plunger to filter. Collect the filtrate in a clean 50 mL Centrifuge Tube (Self-prepared materials) .

Note: Mix immediately after adding Buffer E3. The presence of some white precipitate in the supernatant before filtration is acceptable. When the bacterial biomass is excessively high, it is recommended to extend the centrifugation time to 20-30 min.

6. Add isopropanol equal to 0.3 times the volume of the filtrate. Mix by inverting and apply the mixture to a Spin Column CP1 placed in a 50 mL Collection Tube. Centrifuge at 8,000 rpm for 2 min at room temperature. Discard the flow-through and place the Spin Column CP1 back into the Collection Tube.

Note: The maximum binding capacity of the Spin Column CP1 is 15 mL. Load the solution in multiple steps, keeping the volume applied to the Spin Column CP1 ≤10 mL per load to prevent leakage.

7. Add 10 mL of Buffer PW (ensure ethanol has been added) to the Spin Column CP1. Centrifuge at 8,000 rpm for 2 min at room temperature. Discard the Buffer PW and place the Spin Column CP1 back into the Collection Tube.

8. Repeat Step 7 once.

9. Add 3 mL of anhydrous ethanol to the Spin Column CP1. Centrifuge at 8,000 rpm for 2 min at room temperature. Discard the ethanol.

10. Place the Spin Column CP1 back into the Collection Tube. Centrifuge at 8,000 rpm for 5 min at room temperature to remove residual ethanol.

Note: Air-dry the Spin Column CP1 at room temperature for 10 min to evaporate any residual ethanol. Residual ethanol can interfere with downstream enzymatic reactions.

11. Place the Spin Column CP1 into a clean 50 mL Collection Tube. Apply 1-2 mL of Buffer TB to the center of the Spin Column CP1. Incubate at room temperature for 5 min. Centrifuge at 8,000 rpm for 5 min at room temperature. Transfer all Buffer TB from the 50 mL Collection Tube to a clean 1.5 mL microcentrifuge tube. Store at -20°C.

Note: To increase plasmid recovery efficiency, reload the Buffer TB onto the same column and repeat Step 11. Pre-warming the Buffer TB to 65°C-70°C is recommended. Elution efficiency is highly dependent on pH. If using ddH2O for elution, ensure its pH 7.5-8.0. pH <7.0 significantly reduces efficiency. The optimal Buffer TB volume depends on plasmid copy number and required concentration. The volume of Buffer TB should not be less than 1 mL; an excessively small volume will impair the recovery efficiency.