Gram-positive Bacterium Plasmid Extraction Mini Kit

Cat: D1120
Size: 50T/100T
Storage: Store at room temperature, re-test period for 12 months. **(Note: RNase A and Lysozyme will be shipped as accessory, and store at -20℃)**

Kit Components:

Kit Components	D1120-50T	D1120-100T	Storage
RNase A	100μL	100μL×2	-20℃
Lysozyme	3mL	3mL×2	-20℃
Solution I	15mL	30mL	RT
Solution II	15mL	30mL	RT
Solution III	20mL	40mL	RT
Washing Buffer I	24ml	48ml	RT
Washing Buffer II	15mL	15mL×2	RT
Elution Buffer	15mL	30mL	RT
Adsorption Column	50 units	100 units	RT
Collection Tube	50 units	100 units	RT

Note：Please add absolute ethanol to the Washing Buffer before use. Please refer to the label on the bottle for the added volume. (16mL/32mL of absolute ethanol should be added to each bottle of 50T/100T washing buffer I, and 45mL of absolute ethanol should be added to each bottle of washing buffer II). Add RNaseA to Solution I before use (Add all the RNaseA provided in the kit), mix well, and store at 2-8℃.All centrifugation steps are centrifuged at room temperature using a benchtop centrifuge.

Protocols (*only for reference*):

1. Take 1-5mL bacterial cultures, centrifuge for 1min at 12000rpm, remove supernatant (if there are too much bacterial liquid, bacteria can be collected into a centrifuge tube by multiple centrifugation).
2. Add 200uL Solution I (**check if add RNaseA before use**) to centrifuge tube. Suspend the bacterial cell precipitate thoroughly by pipette or vortex oscillator. Then add 50μL lysozyme to it, mix well, and bathe at 37℃ for more than 30min (the water bath time can be appropriately extended according to the amount of bacterial liquid). Note: If it is not thoroughly mixed, it will affect the lysis and lead to the low amount and purity of the plasmid extraction. If you cannot determine the type of bacteria, please treat as positive bacteria.

3. Add 250 μL Solution II to centrifuge tube, invert and gently rotate the tube 6-8 times to obtain a clear lysate. Note: the mixing must be gentle to avoid contaminating the bacterial genome DNA. The bacterial solution should become clear and thick, and the action time should not exceed 5min,

so as not to damage the plasmid.

4. Add 350uL Solution III to centrifuge tube., immediately and gently invert 6-8 times to mix well, and white flocculent precipitate will appear. Centrifuge at 12000rpm for 10min, transfer the supernatant into a clean tube, please avoid to absorb precipitate. Note: To avoid local precipitation, the solution should be mixed immediately after Solution III is added. If there is a small white precipitate in the supernatant, please centrifugation again.

5. Take the supernatant of the previous step, add 0.4 times the volume of absolute ethanol, and mix well (it can be can be divided twice if there are too much solution).

6. Add the supernatant obtained in the previous step into the adsorption column (the adsorption column is added into the collection tube), put at room temperature for 2 min, centrifuge for 1min at 12000rpm, discard the flow-through liquid, and put the adsorption column back into the collection tube (If it cannot be completed at one time, it can be divided into two adsorption).

7. Add 600uL washing buffer I to adsorption column (**check if add absolute ethanol before use**). Centrifuge at 12000rpm for 1min, discard the solution, place adsorption column back to the collection tube.

8. Add 700uL washing buffer II to adsorption column (**check if add absolute ethanol before use**). Centrifuge at 12000rpm for 1min, discard the solution, place adsorption column back to the collection tube.

9. Add 500uL washing buffer II to adsorption column. Centrifuge at 12000rpm for 1 min, discard the solution, place adsorption column back to the collection tube.

10. Centrifuge the column at 12000rpm for 2min. Open its cap, and put at room temperature or 50℃ for several minutes to remove the rest of washing buffer in adsorption column. Otherwise, the ethanol in the washing buffer will affect subsequent experiments, such as enzyme digestion, PCR, etc.

11. Put adsorption column into a new clean centrifuge tube. Add 50-200uL Elution buffer which is after 65℃ water bath to the center of the adsorption film, put at room temperature for 2min. centrifuge at 12000rpm for 1 min.

12. (Optional)In order to increase the recovery efficiency of plasmids, the eluent obtained can be re-added into the adsorption column, place at room temperature for 2min, and centrifuge at 12000rpm for 1 min.

Notes:

1. Check Solution II, Solution III before use. If the buffer appears turbid, dissolve it by warming at 37℃ for several minutes, they can be used until become clear.

2. If the volume of elution buffer is less than 50uL, it may affect recovery efficiency. The pH value of elution buffer has influence in eluting too. If using water as elution buffer, pH should be controlled at 8.0 (adjusted by NaOH), pH below 7.0 will affect elution efficiency. The extracted DNA should be at -20℃ to prevent degradation.

3. If the extracted plasmid is low copy or larger than 10 kb, please add more bacteria, use 5-10mL overnight culture, while increasing the amount of solution I, solution II and solution III,

appropriately extend the adsorption and elution to increase the extraction efficiency.

4. DNA concentration and purity detection: the size of the extracted plasmid DNA fragments is related to the storage time of the sample, the shear force during the operation and other factors, The recovered DNA fragments can be detected for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. DNA should have a significant absorption peak in OD260, OD260=1 is equal to 50ug/mL double-stranded DNA, 40ug/mL single-stranded DNA. OD260/OD280 should be 1.7-1.9, the value will be lower if using distilled water in eluting, because the pH and ions will affect the light absorption value, but do not show the purity is low.

Related Products:

D1010 6×DNA Loading Buffer
T1060 TAE Buffer,50×
T1050 TBE Buffer,5×
M1060 D2000 DNA Ladder
M1400 1kb DNA Ladder
G8142 GoldView II Nuclear Staining Dyes(5000×)
L1015 LB solid medium (Dry powder)
L1020 SOC Liquid Medium (Dry powder)

Related documents:

[1] F.Yao,X.Y.Xu,Q.Pan,et al. A modified method for plasmid extraction from Lactobacillus plantarum contained lysozyme removal step. Analytical Biochemistry. February 2019;566:37-39. (IF 2.219)

Note: For more information about this product, please refer to Solarbio website.