Plasmid Extraction Mini Kit

Cat: D1100
Size: 50T/100T
Storage: Store at room temperature, re-test period is 12 months. **(Note: RNaseA will be shipped as an accessory, please store at -20℃)**

Kit Components:

Kit Components	D1100-50T	D1100-100T	Storage
RNase A	100μL	100μL×2	-20℃
Solution I	15mL	30mL	RT
Solution II	15mL	30mL	RT
Solution III	20mL	40mL	RT
Washing Buffer	20mL	20mL×2	RT
Elution Buffer	15mL	30mL	RT
Adsorption column (including collection tube)	50 sets	50 sets×2	RT

Introduction:

Plasmid Extraction Mini Kit uses alkaline lysis method to lyse cells, and specifically extract plasmid DNA based on the principle that the centrifugal adsorption column specifically binds to the DNA in the solution under high salt conditions. The silicon matrix material used in the centrifugal adsorption column can adsorb DNA efficiently and specifically, and can remove impurity proteins and other organic compounds in cells to the greatest extent. The plasmid DNA extracted by this kit can be applied to various routine operations, including enzyme digestion, PCR, sequencing, ligation and transformation tests.

Note：Please add absolute ethanol to the washing buffer before use. Please refer to the label on the bottle for the added volume. Add RNaseA to Solution I before use (Add all the RNaseA provided in the kit), mix well, and store at 2-8℃. Unless specified, 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 250uL Solution I **(check if add RNase A before use)** to centrifuge tube. Suspend the bacterial cell precipitate thoroughly by pipette or vortex oscillator. If it is not thoroughly mixed, it will affect the lysis and lead to the low amount and purity of the plasmid extraction.
3. Add 250uL Solution II to centrifuge tube. Invert and gently rotate the tube 6-8 times to fully lyse the bacteria. Note: the mixing must be gentle to avoid contaminating the bacterial genome DNA.

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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 mixture of the previous step into the adsorption column (the adsorption column is added into the collection tube), put for 2min at room temperature, centrifuge at 12000rpm for 1min, discard the solution and place adsorption column back to the collection tube (In order to improve the yield, the solution in the collection tube can be added to the adsorption column once again).

7. Add 750uL washing buffer 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 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. 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.

10. 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 1min.

11. 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 1min.

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. Keep the cover of Solution II, Solution III tightly after use.

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 10kb, 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 DNA Loading Buffer,6×
T1060 TAE Buffer,50×
T1050 TBE Buffer,5×
M1070 D2000 plus DNA Ladder
M1400 1kb DNA Ladder
G8142 GoldView II Nuclear Staining Dyes(5000×)

Related documents:

[1] Deguang Wu,Xuewu Guo,Jun Lu,et al. A rapid and efficient one-step site-directed deletion, insertion, and substitution mutagenesis protocol. Analytical Biochemistry. March 2013;254-258. (IF 2.275)
[2] Jian Dong,Guanglu Wang,Cuiying Zhang,et al. A two-step integration method for seamLess gene deletion in baker's yeast. Analytical Biochemistry. August 2013;30-36. (IF 2.275)
[3] Haigang Tan,Jian Dong,Guanglu Wang,et al. Enhanced freeze tolerance of baker's yeast by overexpressed trehalose- 6-phosphate synthase gene (TPS1) and deleted trehalase genes in frozen dough. Journal of Industrial Microbiology & Biotechnology. August 2014;41. (IF 2.533)

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

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