V02

Free Endotoxin Plasmid Extraction Maxi Kit

Cat: D1150
Size: 10T
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℃;Store endotoxin scavenging agent at room temperature, Store at 2-8℃ after opening the lid)**

Kit Components:

Kit Components	D1150-10T	Storage
RNase A	300μL×2	-20℃
Endotoxin Scavenging Agent	100mL	2-8℃
Solution P1	40mL	RT
Solution P2	40mL	RT
Solution P3	40mL	RT
Washing Buffer	20mL×2	RT
Elution Buffer	20mL	RT
Adsorption Column(including collection tube)	10 sets	RT
collection tube	10 units	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. Endotoxin scavenging agent developed by Solarbio for maximum removal of endotoxins. From 50-100mL of E. coli LB culture medium, 150-250μg high purity plasmid DNA can be quickly extracted, and the extraction rate can reach 85-90%. The plasmid DNA extracted using this kit has a high purity and can be used for a variety of routine operations, including enzyme digestion, PCR, sequencing, linking and transformation tests.

Please add absolute ethanol to the washing buffer before use. Please refer to the label on the bottle for the added volume (one bottle needs to add 60mL absolute ethanol). Solution P1 needs to add RNase A before use (add all the RNase A in the kit), mix well, store at 2-8℃. Unless otherwise specified, all centrifugation steps are centrifuged at room temperature using a benchtop centrifuge.

After opening the lid for the first time, please store the endotoxin scavenging agent at 2-8℃, which can shorten the ice pre-cooling time during use.

Protocols (*only for reference*):

1. Take 50-100mL bacterial cultures, centrifuge for 1min at 11000rpm, remove supernatant (if there are too much bacterial liquid, bacteria can be collected into a centrifuge tube by multiple centrifugation).
2. Add 4mL P1 **(check if add RNase A before use)** to centrifuge tube. Suspend the bacterial cell

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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 4mL P2 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. 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 4mL P3 to centrifuge tube, immediately and gently invert 6-8 times to mix well, and white flocculent precipitate will appear. Centrifuge at 11000rpm 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 P3 is added. If there is a small white precipitate in the supernatant, please centrifugation again.

5. Add 0.2 times volume of the ice pre-cooling endotoxin scavenging agent to supernatant, shake and mix the solution to become cloudy, and bathe in ice for 5-10min until the solution becomes clear.

6. Water bath at 42℃ for 5min. Shock it, solution will be turbid. Centrifuge at 11000rpm for 5minutes at room temperature. The solution should be divided into two phases, the upper water phase containing plasmid DNA and the lower oil phase containing endotoxin.

7. Transfer the upper water phase containing plasmid DNA to the new tube, discard the lower oil phase, and be careful not to inhale the oil phase. Repeat extraction three times, that is, repeat steps 5-7 three times.

8. Add 0.5 times volume of absolute ethanol and mix well, then put in adsorption column **(put adsorption column in collection tube)**, put for 2min at room temperature, centrifuge at 11000rpm for 1min, discard the solution and place adsorption column back to the collection tube.

9. Add 7mL washing buffer to adsorption column **(check if add absolute ethanol before use)**, centrifuge at 11000rpm for 1min, discard the solution, place adsorption column back to the collection tube.

10. Add 7mL washing buffer to adsorption column, centrifuge at 11000rpm for 2min, discard the solution, place adsorption column back to the collection tube.

11. Centrifuge the column at 11000rpm 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.

12. Put adsorption column into a new clean centrifuge tube. Add 1-2mL elution buffer which is after 65℃ water bath to the center of the adsorption film, put at room temperature for 2min. centrifuge at 11000rpm for 2min.

13. 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 11000rpm for 2min.

Notes：

1. Check P2, P3 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 P2, P3 tightly after use.

2. If the volume of elution buffer is less than 500uL, 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 orlarger than 10 kb, please add more bacteria, use 100-200mL overnight culture, while increasing the amount of P1、P2 and P3, 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×
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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