Gram-positive Bacterium Plasmid Extraction Maxi Kit

Cat: D1130

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℃)**

Kit Components:

Kit Components	D1130-10T	Storage
RNase A	300μL×2	-20℃
Lysozyme	10mL	-20℃
Solution I	60mL	RT
Solution II	60mL	RT
Solution III	80mL	RT
Washing Buffer	15mL×2	RT
Elution Buffer	30mL	RT
Adsorption Column	10 units	RT
Collection Tube	20 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 (each bottle needs to add 45mL absolute ethanol separately). Add RNase A to Solution I before use (Add all the RNase A 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 50-200mL bacterial cultures, centrifuge for 5min at 11000rpm, remove supernatant (if there are too much bacterial liquid, bacteria can be collected into a centrifuge tube by multiple centrifugation).
2. Add 5mL Solution I (**check if add RNaseA before use**) and 1mL lysozyme to centrifuge tube. Suspend the bacterial cell precipitate thoroughly by pipette or vortex oscillator, 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.
3. Add 5mL 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 7mL Solution III 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 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 0.5 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 2min, centrifuge for 2min at 11000rpm, 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 7mL washing buffer to adsorption column (**check if add absolute ethanol before use**). Centrifuge at 11000rpm for 2min, discard the solution, place adsorption column back to the collection tube.

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

9. Centrifuge the column at 11000rpm for 5min. 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 1-2mL Elution buffer which is after 65℃ water bath to the center of the adsorption film, put at room temperature for 5min. centrifuge at 11000rpm for 5min and collect the plasmid DNA solution.

11. (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 5min, and centrifuge at 11000rpm for 2min.

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 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 400-800mL 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

本产品仅供科学研究使用。请勿用于临床、诊断、食品、化妆品检测等用途。
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.

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 IINuclear Staining Dyes(5000×)
L1015 LB solid medium (Dry powder)
L1020 SOC Liquid Medium (Dry powder)

Related documents:

[1] Zhongyuan Li,Xiumei Li,Tianhui Liu,et al. The critical roles of exposed surface residues for the thermostability and halotolerance of a novel GH11 xylanase from the metagenomic library of a saline-alkaline soil. International Journal of Biological Macromolecules. July 2019;133:316-323. (IF 4.784)
[2] Liping Du,Lijuan Ma,Qing Ma,et al. Hydrolytic boosting of lignocellulosic biomass by a fungal lytic polysaccharide monooxygenase, AnLPMO15g from Aspergillus niger. Industrial Crops and Products. December 2018;126:309-315. (IF 4.191)

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

Tel: 400-968-6088 https://www.solarbio.com E-mail: sales-china@solarbio.com
Address: No. 85, Liandong U Valley, Middle Zone, 101102, Tongzhou Dist, Beijing, China

本产品仅供科学研究使用。请勿用于临床、诊断、食品、化妆品检测等用途。
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.