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EndoFree Plasmid Maxi Kit

Product Number: PLK1301

Shipping and Storage

1. When used for the first time, add all RNase A carried by the kit to Buffer P1 (final concentration 100ug/ml) and store it at 4℃. If RNase A is inactivated in Buffer P1, the extracted plasmid may be mixed with trace RNA residues. Add RNase A to Buffer P1.
2. When the ambient temperature is low, SDS in Buffer P2 may precipitate and appear turbid or precipitated. It can be heated in a 37℃ water bath for a few minutes to restore clarity. Do not shake violently to avoid excessive foam formation.
3. Avoid volatilization, oxidation and pH change of reagents exposed to air for a long time, and close the cover of each solution in time after use.
4. Buffer ER is transported at room temperature. It can be stored at 4℃ for one month, and stored at -20℃ for a long time.

Components

Component	Storage	PLK1301 10 Preps
RNaseA(10mg/ml)	-20℃	750μl
Buffer P1	4℃	77 ml
Buffer P2	RT	77 ml
Buffer N3	RT	77 ml
Buffer ER	-20℃	25 ml
Buffer PE	RT	63 ml
Buffer WB	RT	25 ml×2
Buffer EB	RT	20 ml
Adsorption column DC	RT	10
Collection pipe (50ml)	RT	10

Description

This kit uses an improved SDS alkaline lysis method to lyse cells. The crude extract is selectively combined with centrifugation to remove endotoxin through a unique buffer Er, and then the silicon matrix membrane in the centrifugal adsorption column selectively binds plasmid DNA in the solution under high salt and low pH, and then removes impurities and other bacterial components through buffer WB. Finally, the pure plasmid DNA is eluted from the silicon matrix membrane by buffer EB with low salt and high pH.

Features

1. The silicon matrix membranes in the centrifugal adsorption column are all specially made adsorption membranes imported from world-famous companies, with minimal difference in adsorption capacity between columns and good repeatability. It overcomes the disadvantage of unstable quality of domestic kit membrane.
2. There is no need to use toxic phenol, chloroform and other reagents, nor ethanol precipitation. It is fast and convenient. 0.5-2mg of pure high copy plasmid DNA can be rapidly extracted from 150-300 ml of Escherichia coli lb (Luria BERTANI) culture solution, and the extraction rate is 80-90%.
3. The unique process formula can remove endotoxin, and the endotoxin content is very low (<0.1eu/μg DNA), and the cell transfection effect was excellent. It can also be directly used in enzyme digestion, transformation, PCR, in vitro transcription, sequencing, and other molecular biology experiments.

Note

1. All centrifugation steps are completed at room temperature unless otherwise specified. A table centrifuge with a 50 ml rotating head and a rotating speed of 12000×g can be used.
2. The amount of plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number. If the plasmid is a low copy plasmid or a large plasmid larger than 10KB, the amount of bacteria should be increased, and the amount of P1, P2, N3 should be increased proportionally. Buffer EB should be preheated at 70 ℃. The adsorption and elution time can be appropriately extended to increase the extraction efficiency.
3. The concentration and purity of the obtained plasmid DNA can be detected by agarose gel electrophoresis and UV spectrophotometer. An od260 value of 1 corresponds to approximately 50μg/ml DNA. The electrophoresis may be a single band or two or more DNA bands, which is mainly caused by the different swimming positions of the supercoiled conformation plasmids in different degrees, and is related to the length of culture time of the extract and the intensity of operation during extraction. The company's products can basically exceed 90% supercoiled under normal operation.
4. The exact molecular size of plasmid DNA can only be known by comparing the DNA molecular weight marker after enzymatic tangent characterization. The swimming position of plasmids in circular or supercoiled state is uncertain, and its exact size cannot be known by electrophoresis.
5. Buffer EB does not contain chelator EDTA and does not affect downstream enzyme digestion, ligation and other reactions. Water elution can also be used, but it should be ensured that the pH is greater than 7.5, and too low pH affects the elution efficiency. Elute with water and the plasmid should be stored at -20°C. If plasmid DNA needs to be stored for a long time, it can be eluted with te Buffer (10mm Tris HCl, 1mm EDTA, pH 8.0), but EDTA may affect the downstream enzyme digestion reaction, which can be diluted appropriately when used.

Protocol(Please read the precautions before the experiment)

Tips: 1) before the first use, please add 100 ml of absolute ethanol into two Buffer WB bottles respectively and mix well. After adding, please tick the box to mark that ethanol has been added in time, so as to avoid adding more than once!

2) Add all RNase A into Buffer P1, mix well, and store at 2-8 ℃ after each use.

1. Take 150-200 ml (no more than 300 ml) of overnight cultured bacterial solution, 12000×g (about 10000rpm), centrifuge for 1-2 minutes, drain the supernatant as much as possible, and collect the bacteria.

   Collect more than 50ml of bacterial liquid. After centrifuging and discarding the supernatant, add more bacterial liquid into the same 50ml tube. Repeat step 1 until enough bacteria are collected.
2. Resuspend the bacterial pellet with 7.5 ml Buffer P1, pipette blow or vortex oscillation to complete suspension.

   If there is a bacteria block that is not thoroughly mixed, it will affect the lysis, resulting in low extraction amount and purity.
3. Add 7.5 ml of Buffer P2, gently flip it up and down for 6-8 times to fully lyse the bacteria, and place it at room temperature for 4-5 minutes.

   Mix gently and do not shake violently to avoid genomic DNA shearing and breaking! It should not take more than 5 minutes! To avoid plasmid damage. At this time, the bacterial liquid should become clear and viscous. If it is muddy, it may be due to too many bacteria and incomplete lysis, so the amount of bacteria should be reduced.
4. Add 7.5ml Buffer N3, turn it up and down gently for 6-8 times immediately, mix well, and white flocculent sediment will appear at this time. Centrifuge at 12000×g for 10-15 minutes and carefully take the supernatant to a new tube to avoid absorbing floating white sediment.

   After adding Buffer N3, it should be mixed immediately to avoid local precipitation of SDS.
5. Add 0.1 volume (10% of the volume of supernatant, about 2.4ml) of Buffer ER to the supernatant obtained in the previous step, rotate upside down and mix well, put it in ice bath or insert it into crushed ice (or refrigerator freezer) for 5 minutes until the turbidity becomes clear and transparent (or still slightly turbid), and mix occasionally in the middle.

   After Buffer ER is added to the supernatant, the supernatant will become turbid, but it should be clear (or slightly turbid) after ice bath.

6. Place it at room temperature for 3-5 minutes, and the solution will soon become turbid when the temperature returns to room temperature, and mix it upside down.
   If the indoor temperature is low or you want to speed up, you can take a water bath at 37-42 ℃, which will soon become muddy and mix upside down.
7. Centrifuge at 8000-10000×g for 10 min at room temperature for phase separation. The upper aqueous phase contains DNA, and the lower blue oily phase contains endotoxin and other impurities. Transfer the upper aqueous phase containing DNA to a new tube (pay attention not to absorb the blue oily layer, which contains impurities such as endotoxin), and discard the oily layer.
8. Add **0.5 volume of isopropanol (about 11ml) to the upper aqueous phase and mix it upside down fully. Divide it into several times (no more than 10 ml each time. Due to the large inclination of the centrifuge rotor in individual cases, it is recommended that the volume of solution added to the adsorption column should not exceed 10 ml to prevent liquid leakage)**. Transfer it to the adsorption column DC (the adsorption column is put into the collection tube), centrifuge at 12000×g for 1 minute, and pour out the waste liquid in the collection tube. Until all mixed solutions pass through this adsorption column.
9. Add 10 ml Buffer PE (please check whether absolute ethanol has been added!), centrifuge at 12000×g for 1 minute, and discard the waste liquid.
   In order to remove trace nuclease and other impurities, this step should be added if the strains used are endo strains such as JM series and HB101 or wild-type strains with abundant nuclease content; If the strains used were XL-1 blue, TOP10 and DH5 α If the nuclease content of other defective strains is low, this step can be skipped.
10. Add 10 ml Buffer WB (please check whether absolute ethanol has been added!), centrifuge at 12000×g for 1 minute, and discard the waste liquid. Add 10ml Buffer WB and rinse again.
11. Put the adsorption column DC back into the empty collection tube and centrifuge at the highest speed (preferably greater than 12000×g) for 2 minutes to dry the residual ethanol on the matrix membrane.
    This step is to completely remove the residual ethanol in the adsorption column, which inhibits the downstream reaction and severely reduces the elution efficiency and plasmid yield.
12. Take out the adsorption column DC, put it into a clean centrifuge tube, add 1-2 ml Buffer EB in the middle of the adsorption membrane (Buffer EB can be preheated in 65-70 ℃ water bath in advance to improve the yield), place it at room temperature for 2 minutes, and centrifuge at 12000×g for 1-2 minutes.
    Recommendation: in order to increase the recovery efficiency of plasmids, the obtained solution can be added to the centrifugal adsorption column again, placed at room temperature for 1 minute, and centrifuged at 12000×g for 1-2 minutes. Elution twice can increase the concentration by about 10%.
    The larger the elution volume is, the higher the elution efficiency is. If a higher plasmid concentration is required, the elution volume can be appropriately reduced. However, it should be noted that a too small volume reduces the plasmid elution efficiency and reduces the plasmid yield (the minimum should not be less than 1ml)