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High Pure Plasmid Maxi Kit

Product Number: PLK1201

Shipping and Storage

1. RNase A is stored in ready to use glycerol Buffer and transported at room temperature. After receipt, it is stored at room temperature for at least 6 months at no more than 25℃, 12 months at 4℃, and -20℃ for a long time.
2. When used for the first time, add all RNase A carried by the kit to Buffer P1 (final concentration 100μg/ml) were stored at 4℃. If RNase A is inactivated in Buffer P1, the extracted plasmid may have trace RNA residue. Add RNase A to Buffer P1.
3. 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.
4. 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.

Components

Component	Storage	PLK1201 10 Preps
RNase A(10mg/ml)	-20℃	750μl
Buffer P1	4℃	77 ml
Buffer P2	RT	77 ml
Buffer N3	RT	77 ml
Buffer PE	RT	63 ml
Buffer WB	RT	50 ml
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 silicon matrix membrane in the centrifugal adsorption column selectively binds plasmid DNA in the solution under high salt and low pH conditions. Then impurities and other bacterial components such as endotoxin are removed by Buffer PE and Buffer WB. Finally, the pure plasmid DNA is eluted from the silicon matrix membrane by Buffer EB with low salt and high pH. The extracted plasmid has a high purity and removes most of the endotoxin. In addition to conventional PCR, digestion, transformation and other experiments, it can also be directly used in general transfection experiments such as protoplast transfection.

Features

1. The silicon matrix membranes in the centrifugal adsorption column are all special adsorption membranes, with minimal difference in adsorption capacity between columns and good repeatability. It overcomes the disadvantage of unstable quality of domestic kit membrane.
2. The unique Buffer PE formula can efficiently remove residual nucleases, even strains with rich nucleases such as JM series and HB101 can be easily removed. The plasmid was effectively prevented from being degraded by nucleases.
3. 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 150-300ml of Escherichia coli lb (Luria BERTANI) culture solution, and the extraction rate is about 80%.
4. The obtained plasmid has high yield, high proportion of supercoiles and good purity, and can be directly used in various molecular biology experiments such as enzyme digestion, transformation, PCR, in vitro transcription, sequencing, protoplast

transfection, etc.

Note

1. All centrifugation steps are completed at room temperature unless otherwise specified. A table centrifuge with a 50ml 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℃. It can appropriately extend the adsorption and elution time and improve 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 is equivalent 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 using for the first time, please add the specified amount of absolute ethanol into the Buffer WB bottle and Buffer PE bottle and mix well. After adding, please tick the box in time to mark that ethanol has been added, so as not to add more than once!

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

1. take 150-200 ml (no more than 300 ml) of overnight cultured bacteria 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 7.5ml Buffer P1, pipette blow or vortex oscillation until it is completely suspended.

   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 7.5ml 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! The time should not exceed 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 the floating white sediment.

   After adding Buffer N3, it should be mixed immediately to avoid local precipitation of SDS.
5. Add 0.5 volume of isopropanol (about 10ml) to the supernatant and mix it upside down fully. Divide it into several times (no more than 10 ml each time. Because of 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.

6. Add 10ml 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.

7. Add 10ml 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.

8. The adsorption column DC was returned to the empty collection tube and centrifuged at 12000×g for 3 min to dry the residual ethanol on the matrix membrane. Open the cover and dry at room temperature for 2-3 minutes.

   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.

9. Take out the adsorption column DC, put it into a clean centrifuge tube, add 1-2ml 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 the plasmid concentration is high, the elution volume can be appropriately reduced. However, it should be noted that the too small volume reduces the plasmid elution efficiency and reduces the plasmid yield (the minimum should not be less than 1ml).