High Pure Plasmid Mini Kit

Product Number: PLK0302

Shipping and Storage

1. When using for the first time, add all RNase A from the test sample to Buffer P1 (final concentration 100μg/ml) and store at 2-8°C. If RNase A is inactivated in Buffer P1, there may be trace RNA residue in the extracted plasmid. Adding RNase A to Buffer P1 is sufficient.
2. When the ambient temperature is low, SDS in Buffer P2 may precipitate turbidity or sediment. It can be heated in a 37 °C water bath for a few minutes to restore clarity. Do not shake violently to avoid excessive foam formation.
3. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly after use.

Components

Component	Storage	PLK0302 100 Preps
Balance Buffer	RT	10ml
RNaseA (10mg/ml)	–20°C	250μl
Buffer P1	4°C	25ml
Buffer P2	RT	25ml
Buffer P3	RT	35ml
Buffer PE	RT	31.5ml
Buffer WB	RT	25ml
Buffer EB	RT	15ml
Adsorption column AC	RT	100
Collection tube(2ml)	RT	100

Note:Buffer PE、Buffer WB-Add the specified amount of ethanol according to the instructions before the first use.

Description

This reagent kit uses an improved SDS alkaline lysis method to lyse cells. The silicon matrix membrane in the centrifuge adsorption column selectively binds to plasmid DNA in the solution under high salt and low pH conditions. Then, impurities and other bacterial components are removed through Buffer PE and Buffer WB. Finally, the pure plasmid DNA is eluted from the silicon matrix membrane with low salt and high pH Buffer EB

Features

1. The silicon matrix membranes inside the centrifugal adsorption column are all made by imported world-renowned companies, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. The unique de Buffer PE formula can efficiently remove residual nucleases, even for strains with rich nuclease content such as JM series and HB101, it can be easily removed. Effectively preventing plasmid degradation by nuclease.
3. It is fast and convenient, and does not require the use of toxic reagents such as phenol and chloroform, nor does it require ethanol precipitation. The obtained plasmids have high yield and good purity, and can be directly used for various molecular biology experiments such as enzyme digestion, transformation, PCR, in vitro transcription, sequencing, etc.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a rotational speed of up

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to 13000rpm.

2. The amount of plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number. Generally, high copy plasmids are recommended to be inoculated in LB medium with appropriate antibiotics at a concentration of 1.5-4.5ml. Overnight cultivation for 14-16 hours can extract up to 20μg of pure plasmids. If the extracted plasmid is a low copy plasmid or a large plasmid larger than 10kb, the usage of the bacterial body should be appropriately increased. 5-10ml of overnight culture should be used, and the dosage of P1, P2, and P3 should be increased proportionally. The other steps are the same.
3. The concentration and purity of the obtained plasmid DNA can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. An OD_{260} value of 1 is equivalent to approximately 50μg/ml of DNA. Electrophoresis may consist of a single band, as well as two or more DNA bands, which are mainly caused by varying degrees of superhelical conformational plasmids swimming in different positions, and are related to the length of extract cultivation time and the intensity of extraction operations. Under normal operating conditions, our company's products can basically exceed 90% of the super spiral.
4. The exact molecular size of plasmid DNA can only be determined by comparing DNA molecular weight markers after enzyme tangency. Plasmids in a circular or super spiral state have uncertain swimming positions and their exact size cannot be determined through electrophoresis.
5. Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme digestion, connection, and other reactions. Water can also be used for elution, but it should be ensured that the pH is greater than 7.5, as low pH affects elution efficiency. Plasmids washed with water 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, pH8.0), but EDTA may affect downstream enzyme digestion reactions and can be appropriately diluted when used.

Use of balance buffer

1. Description

During the long-term placement of nucleic acid adsorption silica gel membrane columns, they react with charges/dust in the air and affect their nucleic acid binding ability.After pre-treatment with equilibrium solution, the silica gel column can greatly reduce the hydrophobic groups of the silica gel membrane in the column and improve the binding ability of nucleic acids.Thus improving the recovery efficiency or yield of silicone columns. The equilibrium solution is a strong alkaline solution. If accidentally touched, please clean it with a large amount of tap water.After use, the bottle cap needs to be tightly closed to avoid contact with air. Store at room temperature. During storage, there may be precipitation formation. Please heat to 37 ℃ to completely eliminate the precipitation.

2. Protocol

Take a new silica gel membrane adsorption column and install it in a collection tube, and suck 100μl of equilibrium solution into the column.Centrifuge at 13000rpm for 1 minute, discard the waste liquid from the collection tube, and reposition the adsorption column in the collection tube. At this point, the balance liquid pre-treatment column is completed. Follow the subsequent operating steps.

Protocol(Please read the precautions before the experiment)

Note:1)Before using it for the first time, please add the specified amount of anhydrous ethanol to Buffer WB and Buffer PE, mix well, and mark the box with a check mark indicating that ethanol has been added in a timely manner to avoid adding it multiple times!

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

1. Take 1.5-4.5 ml of overnight cultured bacterial solution, centrifuge at 12000 rpm for 30 seconds, pour out the supernatant as much as possible, and collect the bacterial body.

Collect more than 1.5 ml of bacterial liquid, centrifuge the supernatant and add more bacterial liquid into the same 1.5ml tube. Repeat step 1 until sufficient bacterial bodies are collected.

2. Use 250μl Buffer P1 to resuspend the bacterial precipitate and vortex oscillate until it is completely suspended.

If there is an incompletely mixed bacterial block, it will affect the lysis, leading to low extraction amount and purity.

3. Add 250μl of Buffer P2, gently flip it up and down 6-8 times to fully lyse the bacterial body, and let it stand at room temperature for 4 minutes to mix gently. Do not shake vigorously to avoid DNA cleavage and breakage! The time used should not exceed 5 minutes! To prevent plasmid damage. At this point, the bacterial liquid should become clear and viscous. If there is a small amount of bacterial body, it can be quickly clear and viscous before proceeding to the next step, not necessarily accurate for 5 minutes.
4. Add 350μl Buffer P3 and gently flip it up and down 6-8 times. When thoroughly mixed, white flocculent precipitates will appear. Centrifuge at 13000rpm for 10 minutes and carefully remove the supernatant.

After adding Buffer P3, it should be immediately mixed to avoid local precipitation of SDS

Balance liquid pre-treatment adsorption column: The use of balance liquid pre-treatment for silica gel adsorption is a necessary step, and the specific method can be found in the previous section "About the use of balance liquid".

5. Add the supernatant obtained from the previous step to the adsorption column AC (the adsorption column is placed in the collection tube), centrifuge at 12000 rpm for 30-60 seconds, and discard the waste liquid in the collection tube.

Optional steps: Add 500μl of buffer PE, centrifuge at 12000 rpm for 30-60 seconds, and discard the waste liquid.

This step is to remove trace impurities such as nucleases. If the JM series HB101 or wild-type strains are used and have rich nucleic acid content, this step should be added; If defective strains such as XL-1 Blue, Top10, and DH5α are used and the nuclease content is low, this step can be skipped.

6. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!) and centrifuge at 12000 rpm for 30 seconds to discard the waste liquid.
7. Place the adsorption column AC back into the empty collection tube, centrifuge at 13000 rpm for 2 minutes, and try to remove Buffer EB as much as possible to avoid residual ethanol in Buffer EB inhibiting downstream reactions.
8. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 50-100μl of Buffer EB in the middle of the adsorption membrane (Buffer EB can be better heated in a water bath at 65-70℃ beforehand). Leave at room temperature for 2 minutes and centrifuge at 12000 rpm for 1 minute. If a large amount of plasmid is required, the obtained solution can be re added to the centrifuge adsorption column and centrifuged for 1 minute.
9. **The larger the elution volume, the higher the elution efficiency. If a high concentration of plasmid is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 30. If the volume is too small, it will reduce the efficiency of plasmid elution and reduce plasmid production.**