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High Pure Plasmid Mini Kit

Product Number: PLK0301

Shipping and Storage

1. RNase A is stored in a ready to use glycerol buffer and transported at room temperature. Upon receipt, it should be stored at room temperature not exceeding 25 ℃ for at least 6 months, at 4 ℃ for 12 months, and for long-term storage at -20 ℃.
2. 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℃. 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.
3. When the ambient temperature is low, SDS in Buffer P2 may precipitate turbidity or sediment. 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. 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	PLK0301 50 Preps	PLK0302 100 Preps	PLK0303 200 Preps
Balance Buffer	RT	5 ml	10ml	20 ml
RNaseA (10mg/ml)	4°C	150 μl	250μl	500 μl
Buffer P1	4°C	15 ml	25ml	50 ml
Buffer P2	RT	15 ml	25ml	50 ml
Buffer P3	RT	20 ml	35ml	70 ml
Buffer PE	RT	16 ml	31.5ml	64 ml
Buffer WB	RT	13 ml	25ml	50 ml
Buffer EB	RT	10 ml	15ml	20 ml
Adsorption column AC and Collection tube(2ml)	RT	50	100	200

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

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1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a rotational speed of up 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℃. 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. Column equilibrium: Add 100μl of equilibrium solution to the adsorption column AC, centrifuge at 12000 rpm for 1 minute, discard the filtrate, and set aside for later use.

Note: Balanced solution can enhance the adsorption capacity of silica gel membrane for nucleic acids. Please use the adsorption column treated on the same day.

2. Take 1.5-5 ml of overnight cultured bacterial solution and add it to a 1.5 ml centrifuge tube. Centrifuge at 12000 rpm for 30

seconds, try to dry the supernatant as much as possible, and collect the bacterial cells.

Note: If collecting more than 1.5 ml of bacterial solution, centrifuge and discard the supernatant. Add more bacterial solution into the same 1.5 ml tube and repeat step 1 until enough bacterial cells are collected.

3. Add 250µl Buffer P1 to resuspend the bacterial sediment, and use a pipette to blow or vortex until completely suspended.

   Note: If there are incompletely mixed bacterial blocks, it will affect the lysis and result in lower extraction volume and purity.

4. Add 250µl of Buffer P2, gently flip up and down 6-8 times to fully lyse the bacterial cells, and leave at room temperature for 4-5 minutes.

   Note: Mix gently and do not shake vigorously to avoid genomic DNA cleavage and breakage! The time used should not exceed 5 minutes! To prevent damage to the plasmid. At this point, the bacterial solution should become clear and viscous. If it is very turbid, it may be due to excessive bacterial cells and incomplete lysis, and the bacterial volume should be reduced.

5. Add 350µl Buffer P3 and gently flip it up and down 6-8 times. Mix thoroughly, and white flocculent sediment will appear. Centrifuge at 12000 rpm for 10 minutes, carefully absorb the supernatant and add it to the adsorption column AC (place the adsorption column in the collection tube) to avoid absorbing floating white sediment.

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

6. Centrifuge at 12000 rpm for 1 minute and discard the filtrate.
7. Add 500µl Buffer PE (please check if anhydrous ethanol has been added first!), centrifuge at 12000 rpm for 30 seconds, and discard the filtrate.
8. Add 600µl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000 rpm for 30 seconds, and discard the filtrate. Add 600µl Buffer WB and rinse again, discard the filtrate.
9. Place the adsorption column in the recovery manifold and centrifuge at 12000 rpm for 2 minutes to remove Buffer WB as much as possible to prevent residual ethanol in Buffer WB from inhibiting downstream reactions.
10. Remove the adsorption column and place it in a clean centrifuge tube. Add 50µl-100µl Buffer EB (Buffer EB can be preheated in a water bath at 80°C-90°C to increase yield) to the middle of the adsorption membrane. Leave it at room temperature for 2 minutes, centrifuge at 12000 rpm for 1 minute, and discard the adsorption column.

    Recommendation: To increase the efficiency of plasmid recovery, the obtained solution can be re added to the centrifugal adsorption column, left at room temperature for 1 minute, and centrifuged at 12000 rpm for 1 minute. Washing twice can increase the concentration by about 10%.

    Note: The larger the elution volume, the higher the elution efficiency. If a higher plasmid concentration is required, the elution volume can be appropriately reduced. However, it should be noted that a smaller volume can reduce plasmid elution efficiency and decrease plasmid yield (the minimum should not be less than 30µl).