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High Pure Yeast Plasmid Fast Maxi Kit

Product Number: PLK1901

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 carried by the reagent kit to Buffer YP1 (final concentration 100μg/ml) and store at 4℃. If RNase A is inactivated in Buffer YP1, the extracted plasmid may contain trace amounts of RNA residue. Adding RNase A to Buffer YP1 is sufficient.
3. When the ambient temperature is low, SDS in Buffer YP2 may precipitate and become turbid or precipitate. 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 in a timely manner after use.

Components

Component	Storage	PLK1901 10 Preps
RNase A(10mg/ml)	-20℃	750μl
Lyticase	4℃	1g
Buffer YP1	4℃	75 ml
Buffer YP2	RT	75 ml
Buffer YP3	RT	100 ml
Buffer PE	RT	63 ml
Buffer WB	RT	50 ml
Buffer EB	RT	20 ml
Adsorption column DC	RT	10
Collection tube (50ml)	RT	10

Description

This reagent kit uses an improved SDS alkaline lysis method to lyse cells and combines Lyticase specific digestion of yeast cell walls. It can isolate high-purity plasmid DNA from yeast culture medium within 1 hour. After yeast collection, Lyticase is added to remove the cell wall, followed by alkaline lysis of the cells. The silica matrix membrane in the centrifuge adsorption column selectively binds to plasmid DNA in the solution under high salt and low pH conditions. Impurities and other bacterial components are then removed through Buffer PE and Buffer WB. Finally, the pure plasmid DNA is washed off the silica matrix membrane using low salt and high pH Buffer EB.

Features

1. The silicon matrix membranes inside the centrifugal adsorption column are all made of Adlai specially designed adsorption membranes, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. Not fast, 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

For Research Use Only

1. All centrifugation steps are completed at room temperature unless otherwise specified, using a desktop centrifuge with a speed of at least 12000×g and a 50ml rotating head.
2. Buffer YP3 and Buffer PE contain irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, rinse with plenty of water or physiological saline.
3. Usually, the copy number of yeast plasmids is very low, and the maximum yield of high copy plasmids is usually about 1ug of plasmids extracted every 5 ml of culture. When used for downstream experiments, it is usually recommended to use 1-5μl as a PCR template; 5-10μl is used for transforming Escherichia coli and selecting highly efficient competent cells.
4. The concentration and purity of the obtained plasmid DNA can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. A OD260 value of 1 is equivalent to approximately 50μg/ml of DNA.Electrophoresis may consist of a single band, as well as 2 or more DNA bands, mainly due to varying degrees of superhelix conformational plasmids swimming in different positions, which are related to the length of extract culture time and the intensity of extraction operations. Under normal operating conditions, our company's products can basically exceed 90% of the super spiral.
5. Users need to bring their own Sorbitol buffer (1M sorbitol, 0.1M Na2EDTA, 14 mM) β-Mercaptoethanol. Preparation method: Dissolve 182.2g of sorbitol in 600ml of deionized water, add 200ml of 0.5 Mna2EDTA (pH 8.0), without adjusting the pH value, make up to 1L and store at 4℃. Add 0.1% before use β-Mercaptoethanol (commercialized) β- The molar concentration of mercaptoethanol is generally 14M.
6. When the OD600 value for bacterial concentration detection is generally 1, the number of brewing yeast cells is 1-2×10^7 cells/ml. Due to differences in bacterial strains and spectrophotometers, even with the same number of cells, the OD value varies greatly. The above is for reference only.
7. Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme cleavage, linking, 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 can affect 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, pH 8.0), but EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.

Protocol(Please read the precautions before the experiment)

Tips: 1) Before the first use, please add the specified amount of anhydrous ethanol to the Buffer WB bottle and Buffer PE bottle, mix well, and mark the added ethanol in the box in a timely manner to avoid multiple additions!
2) Add all RNase A to Buffer YP1 and mix well. Store at 2-8℃ after each use.
3) Pre cool Buffer YP3 on ice.
4) Absorb the amount of Sorbitol buffer used and add 0.1% β- Mercaptoethanol, return to room temperature for later use.

1. Take approximately 100-180ml of yeast culture, 12000×g, centrifuge for 1 minute, pour out the supernatant as much as possible, and collect the bacterial cells.

   Collect more than 50ml of bacterial solution. After centrifuging and discarding the supernatant, add more bacterial solution to the same 50ml tube and repeat step 1 until sufficient bacterial cells are collected.
2. Add 10ml Sorbitol buffer and gently blow to resuspend the cells; Add 0.1g Lyticase (dissolve Lyticase in 2ml Sorbitol buffer before use), mix thoroughly upside down, and incubate at 37 ℃ for 1-2 hours to digest the cell wall. Invert several times in the middle to aid digestion.

   If the effect of breaking the wall is not good and leads to low plasmid yield, the dosage of Lyticase can be increased to increase the enzyme working concentration, and the digestion time can also be extended or the temperature can be raised to 45 ℃ to improve the effect. Yeasts that are not suitable for breaking the wall for digestion can choose Lyticase, Zymolase, or other methods such as adding glass bead vortex oscillation, repeated freeze-thaw, etc.
3. 12000×g, centrifuge for 2 minutes, discard the supernatant as much as possible, add 7ml of Buffer YP1 to resuspend the bacterial precipitate, vortex and shake until completely suspended.

   If there are incompletely mixed bacterial blocks, it will affect the lysis, resulting in low extraction amount and purity.
4. Add 7ml of Buffer YP2 and gently flip it up and down 4-7 times to fully lyse the bacterial cells. Let it stand at room

temperature for 4 minutes.

Mix gently and do not shake vigorously to avoid genomic DNA cleavage! 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 are few bacterial bodies, the next step can be taken quickly after the clear and viscous consistency is achieved, not necessarily accurate for 5 minutes. If it does not become clear, it may be due to excessive bacterial growth and incomplete lysis, and the bacterial population should be reduced.

5. Add 10ml of Buffer YP3 and gently flip it up and down 4-7 times. Mix thoroughly and white flocculent precipitates will appear. Centrifuge 12000×g for 10-15 minutes, take the supernatant carefully to avoid absorbing floating white precipitates.

   After adding Buffer YP3, it should be mixed immediately to avoid local precipitation of SDS. If there is still floating white sediment in the supernatant, it can be centrifuged again and taken.
6. **Optional, generally not required:**Centrifuge at 4 ℃, 12000×g for 10-15 minutes, take the supernatant carefully.
7. Add the supernatant obtained from the previous step to the adsorption column DC (no more than 10 ml each time, as the inclination angle of the centrifuge rotor is relatively large in some cases, it is recommended to add a solution volume of no more than 10 ml to the adsorption column to prevent liquid leakage), centrifuge at 12000×g for 1 minute, and discard the waste liquid in the collection tube. If the volume of the supernatant exceeds 20ml, it can be passed through the column multiple times.
8. Add 10ml of Buffer PE (please check if anhydrous ethanol has been added first!), centrifuge 12000×g for 1 minute, and discard the waste liquid.
9. Add 10ml of Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge 12000×g for 1 minute, and discard the waste liquid.
10. Repeat step 9 once.
11. Place the adsorption column DC back into the empty collection tube and centrifuge at the highest speed (preferably greater than 12000×g) for 3 minutes to dry the residual ethanol on the substrate membrane. Open the lid and let it dry at room temperature for 2-3 minutes.

    This step is to completely remove residual ethanol from the adsorption column, which inhibits downstream reactions and severely reduces elution efficiency, thereby reducing plasmid production.
12. Take out the adsorption column DC and place it in a clean centrifuge tube. Add 1ml of Buffer EB to the middle of the adsorption membrane (Preheating Buffer EB in a 65-70℃ water bath can increase yield), leave at room temperature for 2 minutes, and centrifuge 12000×g for 1-2 minutes.

    Recommendation: To increase the recovery efficiency of plasmids, the obtained solution can be re added to the centrifuge adsorption column, left at room temperature for 1 minute, and centrifuged at 12000×g for 1-2 minutes. Washing twice can increase the concentration by about 10%.

    The larger the elution volume, the higher the elution efficiency. If a higher plasmid concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 0.6ml. If the volume is too small, the elution efficiency of plasmids will be reduced, reducing plasmid production.