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Yeast Plasmid Mini Kit

Product Number: PLK0601

Shipping and Storage

1. 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, there may be trace RNA residues in the extracted plasmid. Adding RNase A to Buffer YP1 is sufficient.
2. SDS in Buffer YP2 may precipitate turbidity or precipitate when the ambient temperature is low. 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. To avoid reducing activity and facilitate transportation, Lyticase (2500U) is provided as a freeze-dried powder. After receiving it, it can be briefly centrifuged and dissolved in 0.25ml of sterilized water to prepare 10U/Ur. As repeated freeze-thaw cycles may reduce enzyme activity, it should be immediately packaged and stored according to the amount used each time, and stored at -20℃.
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	PLK0601 50 Preps
RNase A (10mg/m1)	-20℃	150μl
Lyticase	-20℃	2500U
Buffer YP1	4℃	15 ml
Buffer YP2	RT	15 ml
Buffer YP3	RT	20 ml
Buffer PD	RT	25 ml
Buffer WB	RT	15 ml
Buffer EB	RT	15 ml
Adsorption column AC	RT	50
Collection tube (2ml)	RT	50

Description

This reagent kit uses an improved SDS alkaline lysis method to lyse cells and combines lysase specific digestion of yeast cell walls to isolate high-purity plasmid DNA from yeast culture medium within 1 hour. After yeast collection, wall breaking enzymes are 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 PD and Buffer WB. Finally, pure plasmid DNA is washed off the silica matrix membrane using low salt and high pH Buffer EB.

Features

1. The silicon matrix membrane inside the centrifugal adsorption column is entirely made of specially designed adsorption membranes from world-renowned imported companies, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. Fast and convenient, without the need for toxic reagents such as phenol and chloroform, and without the need for 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.

Application

Suitable for small-scale plasmid preparation in yeast

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm, such as Eppendorf5415C or a similar centrifuge.
2. Buffer YP3 and Buffer PD contain irritating compounds. Latex gloves should be used 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 around 1Ug of plasmids extracted every 5ml 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 mMβ-mercaptoethanol). Preparation method: Dissolve 182.2 grams of sorbitol in 600 ml of deionized water, add 200ml of 0.5M Na2EDTA (pH 8.0), without adjusting the pH value, make up to 1L and store at 4℃. Before use, add 0.1% β-mercaptoethanol (the molar concentration of commercialized β-mercaptoethanol is generally 14M).
6. When the OD600 value for bacterial concentration detection is generally 1, the concentration 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 the integrator 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)

Tip: 1) Before the first use, please add 60ml anhydrous ethanol to 15ml Buffer WB and mix well. After adding, please mark the box with a check mark indicating that ethanol has been added in a timely manner to avoid multiple additions!
2) Add all RNase A to Buffer YP1, mix well, and store at 2-8℃ after each use.
3) Pre cool the YP3 solution 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 1.5-5ml of yeast culture (not exceeding 5×10^7cells), centrifuge at 9000rpm for 30 seconds, discard the supernatant as much as possible, and collect the bacterial cells.
   Collect more than 1.5ml of bacterial solution. After centrifuging and discarding the supernatant, add more bacterial solution to the same 1.5ml tube and repeat step 1 until sufficient bacterial cells are collected.
2. Add 600μl Sorbitol buffer and gently blow to resuspend the cells; Lyticase can be added in a ratio of 20-50U/1×10^7cells (usually 5ml of culture may need to be added to 200U), thoroughly invert and mix, incubate at 37 ℃ for at least 30 minutes to digest the cell wall, and invert several times to aid digestion.
   If the wall breaking effect 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 be extended to improve the effect. Yeasts that are not suitable for Lyticase digestion can choose Zymolase or other methods such as glass bead vortex and repeated

freeze-thaw.

3. Centrifuge at 13.000rpm for 1 minute, discard the supernatant as much as possible, and add 250μl Buffer YP1 to resuspend the bacterial precipitate by vortex oscillation until completely suspended.
   If there are incompletely mixed bacterial blocks, it will affect the lysis, resulting in low extraction amount and purity.
4. Add 250μl of Buffer YP2, gently flip up and down 4-7 times to fully lyse the bacterial cells, and leave 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.
5. Add 350μl Buffer YP3 and gently flip it up and down 4-7 times. When thoroughly mixed, white flocculent precipitates will appear. Let it stand on ice for 3-5 minutes, centrifuge at 13000rpm for 10 minutes, and carefully remove the supernatant.
   After adding Buffer YP3, it should be mixed immediately to avoid local precipitation of SDS. If there are still small white precipitates in the supernatant, the supernatant can be taken after centrifugation again.
6. 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.
7. **Optional steps:** Add 500μl Buffer PD, centrifuge at 12000rpm for 30-60 seconds, and discard the waste liquid. This step is to remove trace amounts of nucleases and other impurities. **If the strains used are JM series, HB101 and other endA strains or wild-type strains with abundant nuclease content, this step should be added; If the strains used are defective strains such as XL-1Blue and DH5a, and the nuclease content is low, this step can be skipped.**
8. Add 700μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30-60 seconds, and discard the waste liquid.
9. Add 700μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30-60 seconds, and discard the waste liquid.
10. Add 500μl Buffer WB, centrifuge at 12000rpm for 30-60 seconds, and discard the waste liquid. Put the adsorption column AC back into the empty collection tube, centrifuge at 13.000rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.
11. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100μl of Buffer EB to the middle of the adsorption membrane (Buffer EB is better heated in a 65-70 ℃ water bath beforehand). Leave at room temperature for 2 minutes and centrifuge at 13.000rpm 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.
    The larger the elution volume, the higher the elution efficiency. If a high plasmid concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 50μl. If the volume is too small, it will reduce the efficiency of plasmid elution and reduce plasmid production.