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BAC/PAC Large Scale Plasmid Extraction Kit

Product Number: PLK2001

Shipping and Storage

1. When using for the first time, add all RNaseA carried by the reagent kit to Buffer P1 (final concentration 100μg/ml) and store at 4℃. If RNaseA is inactivated in Buffer P1, the extracted plasmid may contain trace amounts of RNA residue. Adding RNase A to Buffer P1 is sufficient.
2. Buffer ER can be stored at 4℃ for one month. If it needs to be stored for a long time, it is recommended to store it at -20℃!
3. When the ambient temperature is low, SDS in Buffer P2 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	PLK2001 20Preps
RNaseA(10mg/ml)	-20℃	1.3ml
Buffer P1	4℃	130m1
Buffer P2	RT	100 ml
Buffer P3	RT	110 ml
Buffer IRA	RT	3 ml
Buffer IRB	RT	30 ml
Buffer ER	-20℃	10 ml

Description

The conventional centrifugal column plasmid extraction kit is not suitable for the extraction of large plasmid DNA such as BAC/PAC/P1/Cosmid. This is mainly because the molecular weight of large plasmid DNA is very large, often exceeding 100kb, and generally has low copy number and low yield. The use of conventional centrifugal columns to adsorb membranes has low adsorption efficiency and yield, and passing the membrane will interrupt these large molecular weight plasmid DNA. This kit uses an improved alkaline lysis method to extract plasmid DNA from cultured bacteria. With a unique solution formula and Buffer ER, it only requires a few simple centrifugations to remove impurities such as proteins, polysaccharides, endotoxins, RNA, and obtain high-quality plasmid DNA. The OD260/280 of purified DNA is usually around 1.8, and the resulting plasmid can be directly applied in tasks that require high DNA purity, such as cell transfection and even animal in vivo experiments. The purification process in the later stage is operated in a 1.5ml centrifuge tube, which is simple, does not require special equipment, does not require column passing, and does not require phenol chloroform extraction; Plasmids released by bacterial lysis can be completely recovered without worrying about the loss of plasmid DNA. This method extracts and purifies plasmid DNA with minimal damage to plasmids. Even large plasmids or ultra large BAC/PAC plasmids with a size of 100kb or even 200kb can be effectively purified as long as they can be extracted by alkaline lysis. In addition, the solution type reagents can be scaled up or down in proportion for small/medium/large extraction. Finally, any small volume can be chosen to dissolve the plasmid, with a concentration of up to 3μg/μl.

Features

1. No need to use toxic reagents such as phenol and chloroform, and no need for ethanol precipitation. Rapid and convenient extraction of high-purity BAC plasmid DNA with a typical yield of 30-50μg can be achieved from 150ml of Escherichia coli LB (Luria Bertani) culture medium, with an extraction rate of 80-90%.
2. The obtained plasmids have high yield, high superhelix ratio, and good purity, and can be directly used for various molecular

biology experiments such as transfection, sequencing, and library.

3. The endotoxin content is extremely low (<0.1EU/μg DNA) and can be directly applied to cell transfection

Application

Suitable for the preparation of large plasmids such as BAC/PAC/P1/Cosmid

Note

1. The amount of plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number. If the extracted plasmid is a low copy plasmid or a large plasmid larger than 10kb, the amount of bacterial cells used should be increased, and the amount of P1, P2, and P3 should be increased proportionally.
2. When extracting large plasmids, the operation should be gentle and a suction head with an enlarged opening should be used to prevent mechanical cutting from damaging DNA.
3. After centrifugation of DNA precipitation solution, obvious precipitation may not be visible. If no precipitation is observed and DNA loss is a concern, the supernatant can be retained. After completing all operations, electrophoresis identification can be performed to determine whether the final product has been obtained (hundreds of micrograms of DNA are centrifuged and precipitated on the sidewall of the tube, and obvious clumps may not be visible).
4. The concentration and purity of the obtained plasmid DNA can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. An OD260 value of 1 is equivalent to approximately 50μg/ml 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 95% over rotation.

Protocol

Tip: Add all RNaseA to Buffer P1, mix well, and store at 2-8℃ after use.

1. Take about 150 ml of overnight cultured bacterial solution (maximum not exceeding 180ml-200ml), transfer it into a suitable centrifuge bottle, centrifuge 10000×g at 4℃ for 2 minutes to precipitate the bacterial body, and completely discard the supernatant.
2. Add 5ml of Buffer P1 and thoroughly suspend and shake the bacterial precipitate to completely disperse until there are no flocs present.

   Transfer the bacterial suspension into a 50 ml centrifuge tube and let it sit at room temperature for 3-5 minutes.

   If there are incompletely mixed bacterial blocks, it will affect the lysis, resulting in low extraction amount and purity.
3. Add 5ml of Buffer P2, gently invert the centrifuge tube 6-8 times, and let it stand at room temperature for 4-5 minutes to completely lyse the bacteria and make the solution transparent.

   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 solution should become clear and viscous. If there are few bacterial cells, they can quickly become clear and viscous before proceeding to the next step, 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.
4. Add 5ml of Buffer P3, immediately invert the centrifuge tube 6-8 times, mix thoroughly until white flocculent material is produced. The above lysis solution was centrifuged at 4℃ for 10-15 minutes at 12000~16000×g. The supernatant was carefully aspirated and transferred into a new 50 ml centrifuge tube.

   After adding Buffer P3, it should be mixed immediately to avoid local precipitation of SDS.
5. Add 10 ml of isopropanol, invert the centrifuge tube, and mix thoroughly.

   Note: When using isopropanol precipitation, there is less co precipitation of protein impurities and salt ions, and there may not be obvious large clump precipitation. However, the plasmid can still be completely precipitated, which does not affect the actual plasmid yield. If you are accustomed to seeing larger precipitate clumps, you can choose to precipitate

with 2 times the volume of anhydrous ethanol.

6. Centrifuge at 4℃ for 10 minutes at 12000~16000×g, carefully discard the supernatant, pour it upside down on absorbent paper, gently drain the residual liquid, add 3-5 ml of 70% ethanol to rinse once, centrifuge at the highest speed for 5 minutes, discard the supernatant, and air dry the precipitate.

   If DNA precipitation is too dry, DNA will not be completely dissolved. However, if ethanol is not dried and evaporated completely, too much residue will also cause DNA to not be completely dissolved.

   Note: After centrifugation and precipitation with isopropanol, the purity of the plasmid is very high. It may not be visible on the bottom and side walls of the tube, but it does not affect the yield. In the subsequent steps, carefully blow the bottom of the tube and rinse the side walls where the precipitate is located to dissolve the plasmid.

7. Add 1.4ml Buffer P1 to completely dissolve the precipitate clump. Note that although the plasmid precipitate attached to the bottom and side walls of the tube may not be visible, it should be rinsed off by blowing the bottom of the tube and the side wall where the precipitate is located (large plasmids can be dissolved by gently blowing with a wide mouthed straw). Then transfer the plasmid solution into two new 1.5ml centrifuge tubes (each 700μl).

   Optional steps (generally not required): If the strain has abundant RNA and trace RNA residues, the plasmid solution can be incubated at 60℃ for 15 minutes after this step to digest the RNA.

8. Add 55μl of Buffer IRA to each tube, invert and mix thoroughly, then add about 0.1 volume (about 80μl) of pre cooled Buffer ER with ice, invert and rotate 7-10 times (about 30 seconds), and mix thoroughly. Place in an ice bath or on ice for ≥ 5 minutes, occasionally invert and mix a few times in the middle.

   After adding buffer ER to the supernatant, it will become turbid, but it should return to a clear state after ice bath

   Note: If it is not necessary to remove endotoxins for transfection, only 55μl Buffer IRA can be added in this step. Mix well and leave on ice for 5 minutes. After centrifugation, carefully transfer the supernatant into a new tube and proceed directly to step 11.

9. 42℃ water bath, the solution will become turbid again, mix upside down and incubate at 42℃ for 5 minutes.

10. Centrifuge at room temperature of 14000×g for 5 minutes to separate phases (when the temperature is low, the Buffer ER cannot separate phases, so it must be centrifuged at room temperature of at least 20℃ or ensure that the winter rotation temperature is above 20℃). The upper aqueous phase contains DNA, while the lower blue oily phase contains endotoxins and other impurities. Transfer the upper aqueous phase containing DNA to a new tube (be careful not to inhale the blue oily layer, which contains impurities such as endotoxins), and discard the oily layer.

    The solution must be divided into upper and lower phases, otherwise steps 9-10 should be repeated.

11. Add an equal volume of Buffer IRB (about 750μl) to the upper aqueous phase obtained in the previous step, mix gently, centrifuge at 14000×g for 10 minutes at 4℃, discard the supernatant (be careful not to lose DNA), gently add 1ml of 70% ethanol for washing, centrifuge and discard the supernatant twice, and air dry at room temperature for 5-10 minutes until the ethanol completely evaporates.

12. Add an appropriate amount of TE or pure water (50-100μl) to each centrifuge tube to dissolve the precipitate (it can be shaken in a 37℃ water bath to assist dissolution). It should be noted that many plasmid DNA may adhere to the sidewall of the centrifuge tube. Even if it is not visible, the sidewall should be thoroughly blown to dissolve and recover the plasmid DNA.

    The final precipitate can be dissolved in any small volume as needed, resulting in a high concentration of transfected plasmid DNA (up to 3-5μg/μl). If necessary, customers can also choose a larger volume for dissolution.