CYPL20-CHANGYU BAC/PAC Large Plasmid Kit

Catalog number	Package
CYPL2001	20 preps

Storage and shipping: room temperature (except low-temperature components); stable for 12 months. RNase A and the Endotoxin Removal Agent are stored at 4 °C.

Scope

For the preparation of large plasmids such as BAC/PAC/P1/Cosmid.

Kit components

Component	Storage	20 preps (CYPL2001)
RNase A (10 mg/ml)	4 °C	1.3 ml
Buffer P1	4 °C	130 ml
Buffer P2	15-25 °C	100 ml
Buffer PIII	15-25 °C	100 ml
Impurity Removal Agent A	15-25 °C	3 ml
Impurity Removal Agent B	15-25 °C	30 ml
Endotoxin Removal Agent	4 °C	10 ml

Storage

Note: Except for the low-temperature components, the kit is stable for 12 months at room temperature.

Note: RNase A is supplied in a ready-to-use glycerol buffer and shipped at room temperature. After receipt it is stable for at least 6 months at no more than 25 °C, for 12 months at 4 °C, and long term at -20 °C.

Note: Before first use, add all the RNase A supplied with the kit to Buffer P1 (final concentration 100 μg/ml) and store at 4 °C. If the RNase A in Buffer P1 loses activity, a small amount of RNA may remain in the plasmid preparation; add fresh RNase A to Buffer P1.

Note: The Endotoxin Removal Agent is shipped at room temperature, is stable for 12 months at 4 °C and can be stored long term at -20 °C.

Note: At low ambient temperature, SDS in Buffer P2 may precipitate and the solution may become turbid or form a precipitate. Warm the bottle in a 37 °C water bath for a few minutes until it clears; do not shake vigorously, to avoid excessive foaming.

Note: Avoid prolonged exposure of the reagents to air, which causes evaporation, oxidation and pH changes; close each bottle tightly after use.

Description

Conventional spin-column plasmid kits are not suitable for BAC/PAC/P1/Cosmid large plasmid DNA. These large plasmid DNAs have very high molecular weight, often exceeding 100 kb, and generally have low copy number and low yield; adsorption on conventional spin-column membranes is inefficient and gives low yield, and passage through the membrane can break these high-molecular-weight plasmid DNAs. This kit extracts plasmid DNA from cultured bacteria by a modified alkaline lysis method and uses a distinctive solution formulation and endotoxin removal reagent; only a few simple centrifugations are needed to remove proteins, polysaccharides, endotoxin, RNA and other impurities, yielding high-quality plasmid DNA. The OD260/280 of the purified DNA is usually around 1.8, and the plasmid can be used

directly for cell transfection and even in vivo animal experiments, which require very high DNA purity. The later purification steps are all carried out in 1.5 ml centrifuge tubes; the method is simple and requires no special equipment, no column and no phenol-chloroform extraction. Essentially all the plasmid released by bacterial lysis is recovered, so there is no risk of losing plasmid DNA. The method causes little damage to plasmid DNA; even large plasmids of 100 kb or even more than 200 kb, or very large BAC/PAC plasmids, can be purified effectively as long as they can be extracted by alkaline lysis. In addition, the solution-type reagents can be scaled up or down proportionally for mini, midi or maxi preparation, and the plasmid can finally be dissolved in any small volume, with a concentration up to 3 μg/μl.

Features

No toxic phenol or chloroform and no ethanol precipitation are required. Rapid and convenient: 30-50 μg of pure high-copy plasmid DNA can be obtained rapidly from 150-200 ml of E. coli LB (Luria-Bertani) culture, with a recovery of 80-90%.

The plasmid is obtained in high yield and concentration, with a high supercoiled proportion and good purity, and can be used directly for restriction digestion, transformation, PCR, in vitro transcription, sequencing and other molecular biology applications.

The endotoxin content is very low (<0.1 EU/μg DNA); the plasmid can be used directly for cell transfection.

Notes before starting

Note: The amount of plasmid obtained depends on culture density, plasmid copy number and other factors. For low-copy plasmids or large plasmids over 10 kb, increase the amount of cells and scale up the amounts of P1, P2 and PIII proportionally.

Note: Handle large plasmids gently and use pipette tips with widened openings to prevent mechanical shearing of the DNA.

Note: After precipitation and centrifugation, a visible pellet may not be seen. If no pellet is visible and loss of DNA is a concern, keep the supernatant and check it by electrophoresis after completing all steps to confirm whether the final product was obtained (hundreds of micrograms of DNA pelleted on the tube wall may not form a visible mass).

Note: Plasmid DNA can be checked by agarose gel electrophoresis and UV spectrophotometry; an OD260 of 1 corresponds to about 50 μg/ml DNA. Electrophoresis may show a single band or two or more bands, mainly because supercoiled isoforms migrate differently depending on culture time and the vigor of handling. Under normal handling, supercoiled plasmid accounts for more than 95%.

❖ Procedure

Tip: add all the RNase A to Buffer P1, mix, and store at 2-8 °C after use.

1. Collect 150 ml (not more than 180 ml-220 ml) of overnight culture in a suitable centrifuge bottle and centrifuge at 8,000 ×g (about 9,000 rpm) for 1 min, discarding the supernatant as completely as possible.

If a 50 ml centrifuge tube is used, centrifuge and discard the supernatant, add more culture to the same 50 ml tube and repeat step 1 until sufficient cells have been collected.

2. Add 5 ml Buffer P1 and resuspend the pellet by vigorous shaking until it is completely dispersed and no clumps remain; leave at room temperature for 3-5 min.

Incompletely resuspended cell clumps reduce lysis and lower yield and purity.

3. Add 5 ml Buffer P2, invert the tube gently 6-8 times and leave at room temperature for 5 min to lyse the bacteria completely; the solution becomes clear.

Mix gently; do not vortex, as this shears genomic DNA. Do not exceed 5 min, to avoid damage to the plasmid. The lysate should become clear and viscous; if few cells were used it may clear and become viscous quickly, and the full 5 min is not essential. If the lysate does not clear, too many cells were used or lysis was incomplete, and the amount of

cells should be reduced.

Buffer P2 may lose lysis efficiency on prolonged storage. If plasmid yield drops for no apparent reason, try freshly prepared Buffer P2 (1% SDS, 0.2 M sodium hydroxide), which may improve yield.

4. Add 5 ml Buffer PIII, immediately invert the tube 6-8 times until a white flocculent precipitate forms. Centrifuge the lysate at 12,000-16,000 ×g for 10-15 min, carefully withdraw the supernatant and transfer it to a new 50 ml centrifuge tube.

Mix immediately after adding Buffer PIII to avoid local SDS precipitation.

5. Add 10 ml isopropanol and mix thoroughly by inverting the tube.

Note: isopropanol precipitation co-precipitates less protein impurity and salt, so a large visible pellet may not form, but the plasmid still precipitates completely and the actual yield is unaffected. If a larger visible pellet is preferred, 2 volumes of absolute ethanol can be used instead.

6. Centrifuge at 12,000-16,000 ×g for 10 min, carefully discard the supernatant, drain the tube upside down on absorbent paper, wash once with 3-5 ml 70% ethanol, centrifuge at maximum speed for 5 min, discard the supernatant and air-dry the pellet.

If the DNA pellet is dried too much it will not dissolve completely; however, if the ethanol is not allowed to evaporate, residual ethanol also prevents complete dissolution.

Note: after isopropanol precipitation the plasmid is very pure and may adhere invisibly to the bottom and walls of the tube; this does not affect the yield. In the following steps pipette the bottom and walls of the tube carefully to rinse and dissolve the plasmid.

7. Add 1.4 ml Buffer P1 to dissolve the pellet completely; the plasmid may adhere invisibly to the bottom and walls of the tube, so pipette the bottom and walls carefully (for large plasmids a wide-bore pipette can be used to help dissolution). Then transfer the plasmid solution to 2 new 1.5 ml centrifuge tubes (700 µl each).

Optional step (usually unnecessary): if the strain is rich in RNA and a small amount of RNA remains, incubate the plasmid solution at 60 °C for 15 min at this point to digest the RNA.

8. Add 55 µl Impurity Removal Agent A, mix thoroughly by inversion, then add about 0.1 volume (about 80 µl) of ice-cold Endotoxin Removal Agent, mix thoroughly by inverting 7-10 times (about 30 sec) and place on ice or in an ice bath for at least 5 min, inverting occasionally.

After the Endotoxin Removal Agent is added, the supernatant may become turbid, but it should clear after ice incubation.

Note: if endotoxin removal is not required, add only 55 µl Impurity Removal Agent A at this step, mix thoroughly, leave on ice for 5 min, centrifuge, carefully transfer the supernatant to a new tube and continue directly with step 11.

9. Incubate in a 42 °C water bath until the solution becomes turbid again, mix by inversion and incubate at 42 °C for 5 min.

10. Centrifuge at 14,000 ×g for 5 min at room temperature to separate the phases (below 20 °C the Endotoxin Removal Agent will not separate, so centrifuge at a room temperature of at least 20 °C or keep the rotor above 20 °C in winter). The upper aqueous phase contains the DNA and the lower blue oily phase contains endotoxin and other impurities. Transfer the upper aqueous phase to a new tube (do not take the blue oily layer) and discard the oily phase.

The solution must separate into two phases; otherwise repeat steps 9-10.

11. Add an equal volume of Impurity Removal Agent B (about 750 µl) to the upper aqueous phase, mix gently, centrifuge at 14,000 ×g for 10 min and discard the supernatant (do not lose the DNA); gently add 1 ml 70% ethanol, centrifuge and discard the supernatant, twice in total; drain upside down at room temperature for 5-10 min so that the ethanol evaporates completely.

12. Dissolve the pellet in an appropriate volume of TE or pure water (100-200 µl; incubation in a 37 °C water bath with shaking can assist dissolution). Note that much of the plasmid DNA may adhere to the

walls of the tube; pipette the walls thoroughly even if nothing is visible, to dissolve and recover the plasmid DNA.

The final pellet can be dissolved in any small volume as required, giving transfection-grade plasmid DNA at high concentration (up to 5-10 μg/μl). If desired, a larger volume can be used.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.