CYPL09-CHANGYU Agrobacterium/G+ Plasmid Mini Kit

Catalog number	Package
CYPL0901	50 preps
CYPL0902	100 preps
CYPL0903	200 preps

Storage and shipping: room temperature (except low-temperature components); stable for 12 months. RNase A is stored at 4 °C; Lysozyme is shipped at room temperature and stored at -20 °C.

Kit components

Component	Storage	50 preps (CYPL0901)	100 preps (CYPL0902)	200 preps (CYPL0903)
Equilibration Buffer	15-25 °C	5 ml	10 ml	20 ml
RNase A	4 °C	150 µl	250 µl	500 µl
Lysozyme	-20 °C	0.5 ml	1 ml	2 ml
Buffer AG1	4 °C	15 ml	25 ml	50 ml
Buffer AG2	15-25 °C	15 ml	25 ml	50 ml
Buffer AG3	15-25 °C	20 ml	35 ml	70 ml
Deproteinization Buffer PE	15-25 °C	16 ml	32 ml	64 ml
Wash Buffer WB	15-25 °C	13 ml	25 ml	50 ml
Elution Buffer EB	15-25 °C	10 ml	10 ml	20 ml
Spin column AC	15-25 °C	50 pieces	100 pieces	200 pieces
Collection tube (2 ml)	15-25 °C	50 pieces	100 pieces	200 pieces

Before first use, add absolute ethanol to Wash Buffer WB and Deproteinization Buffer PE as indicated on the bottle labels, mix thoroughly and tick the boxes on the labels to avoid adding ethanol twice.

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: Lysozyme is shipped at room temperature and stored at -20 °C for 12 months after receipt.

Note: Before first use, add all the RNase A supplied with the kit to Buffer AG1 and store at 4 °C, where it is stable for about 3 months. If the RNase A in Buffer AG1 loses activity after prolonged storage, a small amount of RNA may remain in the plasmid preparation; add fresh RNase A to Buffer AG1.

Note: At low ambient temperature, SDS in Buffer AG2 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, then mix again; do not shake vigorously, to avoid excessive foaming.

Description

This kit uses a modified SDS-alkaline lysis method to lyse cells and can extract plasmid DNA from Agrobacterium or Gram-positive bacteria. The silica membrane in the spin column selectively binds plasmid DNA under high-salt, low-pH conditions; impurities and other bacterial components are removed with the deproteinization and wash buffers, and pure plasmid DNA is eluted from the membrane with a

low-salt, high-pH elution buffer.

Features

The silica membrane in the spin column is a specially manufactured adsorption membrane with very little variation between columns and good reproducibility.

A proprietary deproteinization buffer efficiently removes residual nucleases, even from nuclease-rich strains such as the JM series and HB101, preventing degradation of the plasmid.

Rapid and convenient: no toxic phenol or chloroform and no ethanol precipitation are required. The plasmid is obtained in high yield and good purity and can be used directly for restriction digestion, transformation, PCR, in vitro transcription, sequencing and other molecular biology applications.

Notes before starting

Note: All centrifugation steps are performed at room temperature unless otherwise stated, in a benchtop centrifuge capable of 13,000 rpm.

Note: The amount of plasmid obtained depends on culture density, plasmid copy number and other factors. For Agrobacterium plasmids, inoculate a single colony into 1.5-5 ml of LB medium with the appropriate antibiotic and grow overnight for 18-20 hours; 5 ml of culture yields about 5-15 μg of pure plasmid. For low-copy plasmids or large plasmids over 10 kb, use 5-10 ml of overnight culture and scale up the amounts of Buffer AG1, AG2 and AG3 proportionally; the other steps are unchanged.

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 90%.

Note: The exact molecular size of a plasmid must be determined after linearization by restriction digestion and comparison with a DNA molecular weight marker; circular or supercoiled plasmid migrates unpredictably, so electrophoresis cannot give the exact size.

❖ Procedure

Before first use, add absolute ethanol to Wash Buffer WB and Deproteinization Buffer PE as indicated on the bottle labels, mix thoroughly and tick the boxes on the labels to avoid adding ethanol twice. Add all the RNase A supplied with the kit to Buffer AG1 and mix; store at 2-8 °C after each use.

Column equilibration: add 100 μl Equilibration Buffer to spin column AC, centrifuge at 13,000 rpm for 1 min, discard the flow-through and set the column aside. The equilibration buffer enhances nucleic acid binding to the silica membrane; use columns equilibrated on the same day.

1. Collect 1.5-5 ml of overnight culture in a 1.5 ml centrifuge tube, centrifuge at 13,000 rpm for 30 sec and discard the supernatant as completely as possible to collect the cells.

If more than 1.5 ml of culture is needed, centrifuge and discard the supernatant, add more culture to the same 1.5 ml tube and repeat step 1 until sufficient cells have been collected.

2. Add 250 μl Buffer AG1 and resuspend the cell pellet by pipetting or vortexing until completely suspended.

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

3. Add 5 μl Lysozyme solution, mix and incubate at 37 °C for 30 min.

For aged, hard-to-lyse Agrobacterium, the amount of Lysozyme may be increased to 10 μl; it is recommended not to exceed 10 μl.

For hard-to-lyse, thick-walled Gram-positive bacteria with poor extraction results, lysozyme powder may be added to a final concentration of 5-10 mg/ml.

4. Add 250 µl Buffer AG2, mix gently by inverting 6-8 times to lyse the cells, and leave at room temperature for 4-5 min.

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 it remains turbid, too many cells were used or lysis was incomplete, and the amount of cells should be reduced.

5. Add 350 µl Buffer AG3, immediately mix gently by inverting 6-8 times; a white flocculent precipitate forms. Centrifuge at 13,000 rpm for 10 min.

Mix immediately after adding Buffer AG3 to avoid local SDS precipitation.

If speed is the priority, the centrifugation can be shortened to 2 min. A small amount of fine white precipitate in the supernatant does not affect the experiment; if a large amount is present, centrifuge again and take the supernatant.

6. Carefully transfer the supernatant to spin column AC placed in a collection tube, avoiding the floating white precipitate. Centrifuge at 13,000 rpm for 30 sec and discard the flow-through.

Accidental uptake of a small amount of floating white precipitate does not affect the result; it is removed during the subsequent filtration and wash steps.

7. Optional step: add 500 µl Deproteinization Buffer PE (check that absolute ethanol has been added), centrifuge at 13,000 rpm for 15 sec and discard the flow-through.

This step removes trace nucleases and other impurities. It can be omitted for endA-deficient, low-nuclease strains such as XL-1 Blue, Top10 and DH5α. For endA+ strains such as the JM series and HB101, or wild-type strains with high nuclease content, residual nucleases may degrade the plasmid or cause it to smear during downstream digestion, so this step should be performed.

8. Add 600 µl Wash Buffer WB (check that absolute ethanol has been added), centrifuge at 13,000 rpm for 15 sec and discard the flow-through. Repeat with another 600 µl Wash Buffer WB.

9. Return the column to the collection tube and centrifuge at 13,000 rpm for 2 min to remove the wash buffer as completely as possible; residual ethanol inhibits downstream reactions.

10. Place the column in a clean centrifuge tube and apply 30-50 µl Elution Buffer EB to the centre of the membrane; leave at room temperature for 2 min and centrifuge at 13,000 rpm for 1 min.

An elution volume of at least 30 µl is recommended; smaller volumes reduce nucleic acid recovery.

The following steps can help increase DNA concentration: prewarm Elution Buffer EB in an 80-90 °C water bath before elution; reload the first eluate onto the column, leave for 1 min at room temperature and centrifuge at 13,000 rpm for 1 min. A second elution increases the concentration by about 10%.

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