CYPL15-CHANGYU HighYield Plasmid Mini Kit (Low Endotoxin)

Catalog number	Package
CYPL1502	100 preps
CYPL1503	200 preps

Storage and shipping: room temperature; stable for 12 months at room temperature. RNase A is shipped at room temperature and stored at 4 °C.

Kit components

Component	Storage	100 preps (CYPL1502)	200 preps (CYPL1503)
Equilibration Buffer	15-25 °C	10 ml	20 ml
RNase A	4 °C	250 µl	500 µl
Buffer P1	4 °C	25 ml	50 ml
Buffer P2	15-25 °C	25 ml	50 ml
Buffer N3	15-25 °C	25 ml	50 ml
Deproteinization Buffer PE	15-25 °C	32 ml	64 ml
Wash Buffer WB	15-25 °C	25 ml	50 ml
Elution Buffer EB	15-25 °C	15 ml	20 ml
Spin column AC	15-25 °C	100 pieces	200 pieces
Collection tube (2 ml)	15-25 °C	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: 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 and store at 4 °C, where it is stable for about 3 months. If the RNase A in Buffer P1 loses activity after prolonged storage, a small amount of RNA may remain in the plasmid preparation; add fresh RNase A to Buffer P1.

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, then mix again; do not shake vigorously, to avoid excessive foaming.

Description

This kit uses a distinctive high-yield SDS-alkaline lysis formulation to lyse cells, increasing plasmid yield 1-2 fold. The silica membrane in the spin column selectively binds plasmid DNA under high-salt, low-pH conditions; impurities and other bacterial components such as endotoxin 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. The plasmid is very pure and most endotoxin has been removed; besides routine PCR, digestion and transformation, it can be used directly for ordinary transfection such as protoplast transfection. For transfection of demanding, difficult cell lines, the EndoFree plasmid midi/maxi kits are recommended.

Features

Specially improved high-yield buffer formulations increase plasmid yield 1-2 fold.

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 12,000 rpm.

Note: The amount of plasmid obtained depends on culture density, plasmid copy number and other factors. For high-copy plasmids, inoculate a single colony into 1.5-4.5 ml of LB medium with the appropriate antibiotic and grow overnight for 14-16 hours; up to 30-50 μg of pure plasmid can be obtained. For low-copy plasmids or large plasmids over 10 kb, use 5-10 ml of overnight culture and scale up the amounts of Buffer P1, P2 and N3 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 to Buffer P1 and mix; store at 2-8 °C after each use.

1. Collect 1.5-5 ml of overnight culture in a 1.5 ml centrifuge tube, centrifuge at 12,000 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 P1 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 250 μl Buffer P2, 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.

4. Add 250 μl Buffer N3, immediately mix gently by inverting 6-8 times; a white flocculent precipitate forms. Centrifuge at 12,000 rpm for 10 min and carefully transfer the supernatant to a new tube, avoiding the floating white precipitate.

Mix immediately after adding Buffer N3 to avoid local SDS precipitation.

Column equilibration: add 100 µl Equilibration Buffer to spin column AC, centrifuge at 12,000 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.

5. Add 0.5 volume of isopropanol (about 335 µl) to the supernatant from step 4, mix thoroughly by inversion, then load the mixture onto the column in several portions (no more than 750 µl each), centrifuging at 12,000 rpm for 30 sec and discarding the flow-through, until all the mixture has passed through the column.

6. Optional step: add 500 µl Deproteinization Buffer PE (check that absolute ethanol has been added), centrifuge at 12,000 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.

7. Add 600 µl Wash Buffer WB (check that absolute ethanol has been added), centrifuge at 12,000 rpm for 15 sec and discard the flow-through. Repeat with another 600 µl Wash Buffer WB.

8. Return the column to the collection tube and centrifuge at 12,000 rpm for 2 min to remove the wash buffer as completely as possible; residual ethanol inhibits downstream reactions.

9. Place the column in a clean centrifuge tube and apply 50-100 µl Elution Buffer EB to the centre of the membrane; leave at room temperature for 2 min and centrifuge at 12,000 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 12,000 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.