CYPL12-CHANGYU HighYield Plasmid Maxi Kit (Low Endotoxin)

Catalog number	Package
CYPL1201	10 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	10 preps (CYPL1201)
RNase A (10 mg/ml)	4 °C	750 µl
Buffer P1	4 °C	77 ml
Buffer P2	15-25 °C	77 ml
Buffer N3	15-25 °C	77 ml
Deproteinization Buffer PE	15-25 °C	64 ml
Wash Buffer WB	15-25 °C	50 ml
Elution Buffer EB	15-25 °C	20 ml
Spin column DC	15-25 °C	10 pieces
Collection tube (50 ml)	15-25 °C	10 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 (final concentration 100 µg/ml) and store at 4 °C, where it is stable for about 3 months. 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: 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.

No toxic phenol or chloroform and no ethanol precipitation are required. Rapid and convenient: 0.5-2 mg of pure high-copy plasmid DNA can be obtained rapidly from 150-300 ml of E. coli LB (Luria-Bertani) culture, with a recovery of 80-90%.

Notes before starting

Note: All centrifugation steps are performed at room temperature unless otherwise stated, at 8,000 ×g (about 9,000 rpm) in a benchtop centrifuge with a 50 ml rotor.

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 N3 proportionally; prewarm the elution buffer to 70 °C. The adsorption and elution times can be extended appropriately to improve recovery.

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 150-200 ml (not more than 300 ml) of overnight culture in a 50 ml centrifuge tube 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 7.5 ml 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 7.5 ml 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 7.5 ml Buffer N3, immediately mix gently by inverting 6-8 times; a white flocculent precipitate forms. Centrifuge at 8,000 ×g 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.

If white precipitate is floating, push the foam aside with a pipette tip and draw from below the surface; accidental uptake of a small amount of floating white precipitate does not affect the result and it is removed during the subsequent filtration and wash steps.

5. Add 0.5 volume of isopropanol (about 10 ml) to the supernatant, mix thoroughly by inversion, then load the mixture onto spin column DC in several portions; centrifuge at 8,000 ×g for 1 min and discard the flow-through.

With some centrifuges the rotor angle is large; it is recommended not to load more than 10 ml onto the column at a time to avoid leakage. Continue until all the mixture has passed through the column.

6. Optional step: add 10 ml Deproteinization Buffer PE (check that absolute ethanol has been added), centrifuge at 8,000 ×g for 1 min 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 10 ml Wash Buffer WB (check that absolute ethanol has been added), centrifuge at 8,000 ×g for 1 min and discard the flow-through. Repeat with another 10 ml Wash Buffer WB.

8. Return the empty column to the collection tube and centrifuge at 8,000 ×g for 3 min to dry residual ethanol from the membrane.

This step completely removes residual ethanol from the column; residual ethanol inhibits downstream reactions and lowers elution efficiency and plasmid yield.

9. Place the column in a new 50 ml centrifuge tube, air-dry at room temperature for 2-3 min, apply 1-2 ml Elution Buffer EB to the centre of the membrane (prewarmed in a 65-75 °C water bath to improve yield), leave at room temperature for 2 min and centrifuge at 8,000 ×g for 1 min; discard the column.

To increase recovery, reload the eluate onto the spin column, leave at room temperature for 1 min and centrifuge at 8,000 ×g for 1 min. A second elution increases the concentration by about 10%.

The larger the elution volume, the higher the elution efficiency. To obtain a higher plasmid concentration the elution volume can be reduced, but note that too small a volume lowers elution efficiency and plasmid yield (not less than 0.8 ml).

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