CYPL22-CHANGYU EndoFree Plasmid Midi Kit

Catalog number	Package
CYPL2201	20 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; the Endotoxin Removal Agent is stored at 4 °C.

Kit components

Component	Storage	20 preps (CYPL2201)
RNase A (10 mg/ml)	4 °C	500 μl
Equilibration Buffer	15-25 °C	10 ml
Buffer P1	4 °C	50 ml
Buffer P2	15-25 °C	50 ml
Buffer N3	15-25 °C	50 ml
Endotoxin Removal Agent	4 °C	18 ml
Deproteinization Buffer PE	15-25 °C	40 ml
Wash Buffer WB	15-25 °C	25 ml
Elution Buffer EB	15-25 °C	20 ml
Spin column MC (5 ml)	15-25 °C	20 pieces
Collection tube (15 ml)	15-25 °C	20 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: The Endotoxin Removal Agent is shipped at room temperature and is stable for 12 months at 4 °C.

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 optimized alkaline lysis is combined with silica-membrane adsorption for specific binding of plasmid DNA; the specially designed solution system removes impurities, proteins and endotoxin, and the proprietary Endotoxin Removal Agent further reduces endotoxin to a minimum. Besides routine PCR, restriction digestion, sequencing and transformation, the plasmid can be used directly for protoplast transfection and eukaryotic cell transfection.

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.

No toxic phenol or chloroform and no ethanol precipitation are required. Rapid and convenient: 100-500 μg of pure high-copy plasmid DNA can be obtained rapidly from 30-70 ml of E. coli LB (Luria-Bertani) culture.

Endotoxin is removed by a proprietary process; the endotoxin content is very low (<0.1 EU/μg DNA) and transfection performance is excellent. The plasmid can also 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, at 8,000 ×g (about 9,000 rpm) in a benchtop centrifuge with a rotor for tubes of 15 ml or larger.

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.

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 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.

1. Collect 30-50 ml (not more than 75 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 15 ml centrifuge tube is used, centrifuge and discard the supernatant, add more culture to the same 15 ml tube and repeat step 1 until sufficient cells have been collected.

2. Add 2.3 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 2.3 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 2.3 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.

Column equilibration: add 0.5 ml Equilibration Buffer to spin column MC, centrifuge at 8,000 ×g 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 0.1 volume (10% of the supernatant volume, about 0.7 ml) of Endotoxin Removal Agent to the supernatant from step 4 and mix by inversion.

6. Add 0.5 volume of isopropanol (about 3.3 ml) to the mixture, mix thoroughly by inversion, then load it onto spin column MC 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 3.5 ml onto the column at a time to avoid leakage. Continue until all the mixture has passed through the column.

7. Optional step: add 3 ml Deproteinization Buffer PE (check that absolute ethanol has been added), centrifuged 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.

8. Add 3 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 3 ml Wash Buffer WB.

9. 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.

10. Place the column in a new 15 ml centrifuge tube, air-dry at room temperature for 2-3 min, apply 0.5 ml Elution Buffer EB (which may be varied from 0.3 ml to 0.6 ml) to the centre of the membrane (prewarmed in a 65-75 °C water bath to improve yield), leave at room temperature for 3 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 0.3 ml).

❖ Appendix: Enhanced endotoxin removal

If the cells to be transfected are delicate, such as primary cells, or are otherwise difficult to transfect, this enhanced endotoxin removal procedure is recommended to remove endotoxin more thoroughly and achieve the best transfection results.

1. Following step 5 of the main procedure, place the supernatant mixed with 0.1 volume of Endotoxin Removal Agent in an ice bath (or a freezer) for 5 min until the turbidity clears to transparent (or remains slightly turbid), inverting once in between.

After the Endotoxin Removal Agent is added, the supernatant may become transiently turbid, but it should clear (or become only slightly turbid) after ice incubation.

2. Leave at room temperature for 3-5 min; as the temperature returns to room temperature the solution quickly becomes turbid again; mix by inversion.

If the room is cold or a faster result is needed, incubate in a 37-42 °C water bath until the solution quickly becomes turbid, then mix by inversion.

3. Centrifuge at room temperature at 8,000 ×g for 10 min to separate the phases. The upper aqueous phase contains plasmid DNA and the lower blue oily phase contains endotoxin and other impurities. Transfer the DNA-containing upper aqueous phase to a new tube (do not take the blue oily layer) and discard the oily phase.

4. Add 0.5 volume of isopropanol (about 3.3 ml) to the supernatant from step 3, mix thoroughly by inversion, then continue with step 6 of the main procedure, loading the mixture onto column MC and completing the remaining steps.

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