NZYMaxiprep

Catalogue numbers:

MB05101, 5 columns
MB05102, 2 x 5 columns
MB05103, 5 x 5 columns

Description

NZYMaxiprep kit is designed for the rapid, large-scale preparation of highly pure plasmid DNA (typically 500 µg) from recombinant *Escherichia coli* strains. Plasmid DNA binds selectively to NZYTech columns charged with a silica-based anion-exchange resin. All contaminants, such as proteins, RNA, salts, nucleotides and oligos (<40-mer) are washed from the column. In the elution step, the positive charge of the resin is neutralized by a pH shift to slightly alkaline conditions and pure plasmid DNA is eluted in a high-salt elution buffer. The purified nucleic acid products are suitable for use in the most demanding molecular biology applications, including transfection, *in vitro* transcription, automated fluorescent or manual sequencing, cloning, PCR and hybridization (Figure 1). To isolate DNA from low copy number plasmids, BACs or cosmids, or to obtain higher DNA concentrations, use 500 mL of *E. coli* cultures and double the volumes of Buffers M1, M2 and M3.

Storage conditions and reagents preparation

All kit components can be stored at room temperature (20-25 °C) and are stable till the expiry date. Before use, dissolve the lyophilized RNase A by the addition of 1 mL of Buffer M1. Pipette up and down until the enzyme is dissolved completely. Transfer the RNase A solution into the Buffer M1 bottle and mix thoroughly. Buffer M1 with RNase A should be stored at 4 °C for frequent use and at -20 °C for infrequent use. Buffer M2 may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37 °C.

System Components

Component	5 columns
Buffer M1	75 mL
Buffer M2	75 mL
Buffer M3	75 mL
Buffer MEQ	35 mL
Buffer MW	2 × 90 mL
Buffer ME	80 mL
RNase A	8 mg
NZYTech Plasmid Maxi Columns	5

Growing of bacterial cultures

LB medium is recommended for the cultivation of bacterial cells. The cell culture should be inoculated from a single colony and incubated at 37 °C with constant shaking (200-250 rpm) preferably for 12-16 hours. Alternatively, rich media like 2×YT or TB may be used. Cells grow faster in these media and reach the stationary phase much earlier than in LB. This may lead to a higher percentage of dead or starving cells when starting the preparation, leading to partially degraded plasmid DNA that might be contaminated with chromosomal DNA. In addition, overgrown cultures may result in too much bacterial material affecting the efficacy of the lysis and precipitation steps. Cell cultures should be grown under antibiotic selection at all times to ensure plasmid propagation.

Protocol for plasmid DNA purification from *Escherichia coli* cells

1. Culture and harvest of bacterial cells

Pellet 100-150 mL of an *E. coli* LB culture by centrifugation for 10 min at 6,000 x*g* under refrigeration conditions (4 °C). Discard supernatant. For low copy number plasmids use 500 mL of cells and double the volume of Buffers M1, M2 and M3. Remove as much media as possible.

2. Cell lysis

Re-suspend cell pellet in 12 mL of Buffer M1, containing RNase A, by vigorous vortexing. Add 12 mL of Buffer M2 and mix gently by inverting the tube for 5 times. Do not vortex. Check Buffer M2 for SDS precipitation before use. Incubate at room temperature (20-25 °C) for 5 min.

3. Neutralization

Add 12 mL of pre-cooled Buffer M3 to the bacterial suspension. Mix gently by inverting the tube for 10-15 times. Do not vortex. The flask or the tube used for this step should not be filled

more than two thirds to allow homogeneous mixing. Immediately proceed with step 5 – an incubation of the lysate is not necessary.

4. Column equilibration

Equilibrate an NZYTech Plasmid Maxi Column with 6 mL Buffer MEQ. Allow the column to empty by gravity flow.

5. Clarification of lysate and column loading

Centrifuge for 30 min at ≥20,000 x*g* at 4°C. If the supernatant still contains suspended matter transfer it to a new tube and repeat the centrifugation for 15 min. Apply the lysate to the equilibrated NZYTech Plasmid Maxi Column. Allow the column to empty by gravity flow.

6. Column washing

Wash the NZYTech Plasmid Maxi Column with 32 mL of Buffer MW. Allow the column to empty by gravity flow.

7. Elution of highly pure DNA

Elute the plasmid DNA with 15 mL of Buffer ME. Allow the column to empty by gravity flow. Collect the eluate in a clean tube.

Note: preheating buffer ME to 50 °C prior to elution may improve yields for large constructs.

8. DNA precipitation

Add 10.5 mL of room-temperature isopropanol (not provided) to precipitate the eluted plasmid DNA. Mix well and let the mixture sit for 2 minutes. Centrifuge at ≥15,000 x*g* for 30 min under refrigeration conditions (4 °C). Carefully discard the supernatant.

9. DNA pellet wash and dry

Add 5 mL of room-temperature 70% ethanol (not provided) to the pellet and centrifuge at ≥15,000 x*g* for 10-15 minutes at room temperature. Carefully remove ethanol completely from the tube with a pipette tip. Allow the pellet to dry at room temperature.

10. DNA reconstitution

Dissolve the DNA pellet in an appropriate volume of Buffer TE or sterile water (both not provided). Determine plasmid yield by UV spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis.

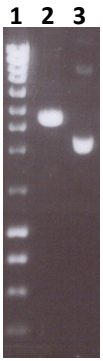

Figure 1. Agarose gel electrophoresis of NZYTech plasmid pNZY28 (2 µL) digested with *Eco*R I (lane 2) or undigested (lane 3). Lane 1 contains NZYDNA Ladder III (MB044).

Quality control assay

All components of NZYMaxiprep kit are tested following the isolation protocol described above. The purification system must isolate 800-1000 µg of pNZY28 plasmid DNA per column.

V1901

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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