MB050_IFU_EN_V2401

NZYMidiprep

Catalogue number	Presentation
MB05003	5 columns

Description

NZYMidiprep kit is designed for the rapid, medium-scale preparation of highly pure plasmid DNA (typically 100 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. To isolate DNA from low copy number plasmids, BACs or cosmids, or to obtain higher DNA concentrations, use 100 mL of *E. coli* cultures and double the volumes of Buffers M1, M2 and M3.

Shipping & Storage Conditions

This product is shipped at room temperature. All kit components can be stored at room temperature (15-25 °C) and are stable till the expiry date if stored as specified.

Components

COMPONENT	MB05003 (5 COLUMNS)
Buffer M1	25 mL
Buffer M2	25 mL
Buffer M3	25 mL
Buffer MEQ	15 mL
Buffer MW	60 mL
Buffer ME	30 mL
RNase A	2 mg
NZYtech Plasmid Midi Columns	5

Reagents, Materials and Equipment Required but Not Provided

- 70% and 96-100% ethanol solutions
- Buffer TE or MB grade sterile water
- Isopropanol
- 1,5 mL microcentrifuge tubes and disposable tips
- Centrifuge for 1,5 mL microcentrifuge tubes

Standard Protocol

Recommendations before starting

- Buffer M2 may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37 °C.

Procedures before starting

Reagents Preparation

- Buffer M1: add 1 mL of Buffer M1 to the RNase A vial and vortex. Transfer the resulting 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.

Procedure

1. Cultivate and harvest bacterial cells

Please see the Technical Notes section for recommendations on bacterial culture growth.

Pellet 25-30 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 100 mL of cells and double the volumes of Buffers M1, M2 and M3. Remove as much media as possible.

2. Cell Lysis

Re-suspend the cell pellet in 4 mL of Buffer M1, containing RNase A, by vigorous vortexing. Add 4 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 2-3 min (max. 5 min).

3. Neutralization

Add 4 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. Columns Equilibration

Equilibrate an NZYtech Plasmid Midi Column with 2.5 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 Midi Column. Allow the column to empty by gravity flow.

6. Column Washing

Wash the NZYtech Plasmid Midi Column with 10 mL Buffer MW. Allow the column to empty by gravity flow.

7. Elution of highly pure DNA

Elute the plasmid DNA with 5 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 3.5 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 2 mL of room-temperature 70% ethanol (not provided) to the pellet and centrifuge at ≥15,000 x *g* for 5 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.

Technical Notes

Growing of bacterial cultures

LB medium is recommended for cultivation of bacterial cells. 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. Pick a single colony from a freshly streaked selective plate and inoculate a culture of 1–5 mL LB medium containing the appropriate selective antibiotic. Incubate for 12–16 h at 37 °C with constant and vigorous shaking (200-250 rpm). Cell cultures should be always grown under antibiotic selection to ensure plasmid propagation.

Quality control assay

All components of NZYMidiprep kit are tested following the isolation protocol described above. The purification system must isolate 80-150 µg of pNZY28 plasmid DNA per column.

Troubleshooting

LOW OR NO DNA YIELD
• **Incomplete lysis/viscous lysate**
Make sure that the culture volumes were used according to the recommendations.
• **Presence of SDS precipitates in the sample**
Check Buffer M2 for SDS precipitation. After finishing the lysis steps, immediately load the lysate onto the NZYtech Plasmid Midi Column.
CLOGGED COLUMNS
• **Large amount of sample material**
Check if the culture volume used is recommended. Do not use a large volume of sample. Increasing culture volumes not only obstruct the column but may also reduce plasmid DNA yields due to inadequate lysis.
LOW DNA QUALITY
• **Presence of RNA**
Make sure that RNAse A was added to Buffer M1. See Reagents preparation described above.
• **Degraded DNA/DNase contamination**
Check your working area and pipettes for possible DNAse contamination. Implement stringent cleanliness protocols.
NO DNA PELLET AFTER PRECIPITATION
• **No precipitation of DNA**
Ensure that was used at least 0.7 volumes of isopropanol (precipitating solvent).
• **Pellet was lost**
Process the precipitate with care. Discard solutions carefully.
DNA IS OPAQUE OR WHITE
• **Co-precipitation of salts**
Verify the purity of isopropanol and ensure that the isopropanol being used is at room-temperature. Resuspend the white/opaque pellet in Buffer MEQ and repeat the DNA precipitation step.

By addressing these possible causes and implementing the suggested solutions, you can troubleshoot common issues encountered during the DNA isolation process and achieve better results with the NZYMidiprep kit.