MB399_IFU_EN_V2401

NZYMaxiprep Endotoxin Free

Catalogue number	Presentation
MB39901	5 columns
MB39902	2 x 5 columns

Description

NZYMaxiprep Endotoxin Free Kit was designed for the rapid, medium-scale preparation of highly pure plasmid DNA (typically 500 µg) from recombinant *Escherichia coli* strains, virtually free of endotoxins (<0.1 EU/µg plasmid DNA). Plasmid DNA binds selectively to NZYMaxi-EF columns charged with a silica-based anion-exchange resin. Effective washes allow the complete removal of contaminants, such as proteins, RNA, salts, nucleotides, oligos and endotoxins. 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 to use in transfection of very sensitive cells like primary or neuronal cells. Using NZYMaxiprep Endotoxin Free Kit, in two efficient washing steps, the plasmid DNA is eluted completely endotoxin free.

Shipping & Storage conditions

This product is shipped at room temperature. All kit components can be stored at room temperature (20-25 °C) and are stable till the expiry date if stored as specified. Buffer NML2-EF may form a precipitate of SDS if the temperature of storage is below 20°C. If salt precipitate is observed, dissolve the precipitate by warming the solution at 37 °C for several minutes.

Components

COMPONENT	MB39901 (5 COLUMNS)
Buffer NML1-EF	70 mL
Buffer NML2-EF	80 mL
Buffer NML3-EF	80 mL
Buffer NMEQ-EF	50 mL
Buffer NMW1-EF	300 mL
Buffer NMW2-EF	150 mL
Buffer NMEL-EF	100 mL
Buffer NME-EF	15 mL
Endotoxin-Free H_2O	15 mL
70% Ethanol (concentrate)	15 mL
RNase A (lyophilized)	7 mg
NZYMaxi-EF Columns	5 units
NZYFolded Filters	5 units
NZYPlastic Washers	2 units

Reagents, Materials and Equipment Required but Not Provided

- 96-100% ethanol
- Isopropanol at room-temperature
- Bench centrifuge

Specifications

Culture Volumes and Expected Plasmid Yield: This protocol was designed for purification up to 500 µg endotoxin-free plasmid DNA using NZYMaxiprep Endotoxin Free Kit. The maximum recommended culture volumes and expected yields for high- and low-copy number plasmids are presented in the following table:

PLASMID TYPE	MAXIMUM CULTURE VOLUME	EXPECTED YIELD
High-copy number	100 mL	300-500 µg
Low-copy number	250 mL	50-250 µg

Column type: NZYMaxi-EF Columns are gravity flow columns and are free of DNases and RNases.

Endotoxin level: < 0.1 EU/µg DNA.

Standard Protocol

Procedures before starting

Reagents Preparation

- Add 1 mL of Buffer NML1-EF to the RNase A vial and pipette up and down until RNAse A is completely dissolved. Transfer the resulting solution into the Buffer NML1-EF bottle and mix thoroughly. Buffer NML1-EF with RNase A should be stored at 2-8 °C for frequent use and from -30 °C to -15 °C for infrequent use.
- Add 35 mL of 96-100% ethanol to the bottle labelled "70% Ethanol".
- Before use, refrigerate the Buffer NML3-EF at 2-8 °C.

Procedure

1. Culture and harvest bacterial cells

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

Pellet 30-100 mL of an *E. coli* LB culture by centrifugation the culture for 15 min at 4,500-6,000 xg under refrigeration conditions (2-8 °C). Discard supernatant.

Note: *For low-copy number plasmids use 250 mL of cells and double the volumes of Buffers NML1-EF, NML2-EF and NML3-EF.*

2. Cell lysis

Resuspend the cell pellet in 12 mL of Buffer NML1-EF, containing RNase A, by vigorous vortexing.

Note: For difficult-to-lyse strains, resuspend the pellet in Buffer NML1-EF supplemented with lysozyme (2 mg/mL final concentration).

Add 12 mL of Buffer NML2-EF to the suspension and mix gently by inverting the tube for 6-8 times. Incubate at room temperature for 2-3 min. Do not vortex.

Note: *Check Buffer NML2-EF for SDS precipitation before use.*

Add 12 mL of pre-cooled Buffer NML3-EF (2-8 °C) to the suspension. Mix the lysate gently by inverting the tube for 6-8 times. Incubate the suspension for 5 min on ice before continuing with "Clarification of the lysate" step.

3. Column equilibration

Equilibrate a NZYMaxi-EF Column with 5 mL of Buffer NMEQ-EF. Allow the column to empty by gravity flow.

4. Clarification of lysate

Place the NZYFolded Filter in a small funnel and pre-wet the filter with a few drops of Buffer NMEQ-EF or sterile H_2O.

Apply the lysate directly onto the wet filter and **collect the flow-through in a clean tube**.

Note: *Alternatively, centrifuge the crude lysate under refrigeration conditions (2-8 °C) for 40 min at 12,000 xg. Carefully remove the supernatant from the white precipitate and apply it onto the equilibrated NZYMaxi-EF Column.*

5. DNA binding

Load the cleared lysate from step 4 onto the NZYMaxi-EF Column. Allow the column to empty by gravity flow.

6. Column washing

Wash the NZYMaxi-EF Column with 2 × 24 mL Buffer NMW1-EF. Allow the column to empty by gravity flow. Discard flow-through.

Wash the NZYMaxi-EF Column with 2 × 12 mL Buffer NMW2-EF. Allow the column to empty by gravity flow. Discard flow-through.

7. DNA elution

Elute the endotoxin free plasmid DNA with 15 mL of Buffer NMEL-EF. Allow the column to empty by gravity flow. Collect the eluate in a clean tube.

Note: *We recommend precipitating the eluate as soon as possible (step 8).*

8. DNA precipitation

Add 11 mL of room-temperature isopropanol (not provided) to precipitate the eluted plasmid DNA and mix carefully. Centrifuge at ≥ 15,000 xg 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 to the pellet. Centrifuge at ≥ 15,000 xg for 15 min at room temperature.

Carefully remove ethanol from the tube with a pipette tip. Air-dry the pellet for 5-10 min.

10. DNA reconstitution

Dissolve the DNA pellet in an appropriate volume of Buffer NME-EF or Endotoxin-Free H_2O.

Determine plasmid yield by UV spectrophotometry and confirm plasmid integrity by agarose gel electrophoresis.

Technical Notes

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 medium. 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 always grown under antibiotic selection to ensure effective plasmid propagation.

Quality control assay

Functional assay

All components of NZYMaxiprep Endotoxin Free Kit are tested following the isolation protocol described above. The purification system must isolate >400 µg of pNZY28 plasmid DNA per column.

Troubleshooting

Troubleshooting is often a systematic, meticulous process where varying one parameter at a time and evaluating impacts can unveil the root cause of issues. These adjusted suggestions, incorporating a blend of specificity and exploratory approaches, aim to enhance the clarity and actionability of your troubleshooting guide. Should any other technical or procedural aspects require attention, your feedback and additional information will always be welcomed.

LOW OR NO PLASMID 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 NML2-EF for SDS precipitation. After finishing the lysis steps, immediately load the lysate onto the NZYMaxi-EF 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.
• **Uncleared lysate**
Check if the NZYFolded Filters were used to clarification of lysate.
LOW DNA QUALITY

• **Presence of RNA**
Make sure that RNAse A was added to Buffer NML1-EF. 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 purity and ensure that the isopropanol used is at room-temperature. Resuspend the white/opaque pellet in Buffer NMEQ-EF and repeat the DNA precipitation step.