NZYMaxiprep Endotoxin Free

Catalogue number:

MB39901, 5 columns
MB39902, 2 × 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 columns-Endotoxin Free 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.

System Components

Component	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 (conc.)	15 mL
RNase A	7 mg
NZYMaxi-EF Columns	5
NZYFolded Filters	5
NZYPlastic Washers	2

Storage conditions and reagents preparation

All kit components can be stored at room temperature (20-25 °C) and are stable till the expiry date. 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.

Before the first use, prepare working solutions as follows:

- 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 should be stored at 4 °C for frequent use and at -20 °C for infrequent use.
- Add 35 mL of 96-100% ethanol to the bottle labelled "**70% Ethanol**".

Safety Instructions

Wear gloves and goggles and follow the safety instructions indicated below.

The following components of NZYMaxiprep Endotoxin free kit contain hazardous contents. According GHS classification, only harmful chemicals/mixtures need not be labelled with H and P phrases until 125 mL or 125 g.

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Buffer NML2-EF	Sodium hydroxide <2%	 Warning	H315, H319	P234, P280, P302+352, P305+351+338, P332+313, P337+313, P390, P406
Buffers NMEQ-EF, NMW1-EF, NMW2-EF, NMEL-EF	Ethanol 5-20%	 Warning	H226	P210, P233, P403+235
RNAse A	RNAse A	 Danger	H317, H334	P261, P280, P302+352, P304+340, P332+313, P342+311, P363

Hazard Phrases

H226	Flammable liquid and vapour.
H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precaution phrases

P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P234	Keep only in original container.
P261	Avoid breathing dust.
P280	Wear protective gloves/eye protection.
P302+352	IF ON SKIN: Wash with plenty of water.
P304+340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+351+338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.
P332+313	If skin irritation occurs: Gel medical advice/attention.
P337+313	Get medical advice/attention.
P342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor/…
P363	Wash contaminated clothing before reuse.
P390	Absorb spillage to prevent material damage.
P403+235	Store in a well-ventilated place. Keep cool.
P406	Store in a corrosive resistant container with a resistant inner liner.

For more information please see Material Safety Data Sheets (www.nzytech.com).

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 effective plasmid propagation.

Protocol for Endotoxin-free plasmid DNA purification

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

1. Culture and harvest bacterial cells

Pellet 30-100 mL of an *E. coli* LB culture by centrifugation the culture for 15 min at 4,500-6,000 x*g* under refrigeration conditions (4 °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.

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 (4 ºC) 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 the 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 for 40 min at 4 ºC, 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. Elution of DNA

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 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 to the pellet. Centrifuge at ≥ 15,000 x*g* 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.

Quality control 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.

V1901

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

For research use only

Notes:

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