NZYSpeedy Miniprep

Catalogue numbers:

MB21001, 50 columns
MB21002, 200 columns
MB21003, 5 x 200 columns
MB21004, 2 x 50 columns

Description

NZYSpeedy Miniprep kit is designed for the ultra-rapid, small-scale preparation of highly pure plasmid DNA from recombinant *Escherichia coli* strains. NZYTech´s speedy miniprep procedure is based on the alkaline lysis of bacterial cells followed by adsorption of DNA onto a new specially treated silica membrane in the presence of high salt. The plasmid DNA is selectively adsorbed in the silica gel-based NZYTech plasmid spin column and other impurities such as proteins, salts, nucleotides and oligos (<40-mer) are washed away. The eluted DNA is suitable for applications like automated fluorescent sequencing, PCR and a wide range of other enzymatic manipulations. NZYSpeedy Miniprep kit includes a newly developed washing buffer (AS) which ensures complete removal of contaminants in a rapid washing step.

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, add 1 mL of Buffer A1 to the RNase A vial and vortex. Transfer the resulting solution into the Buffer A1 bottle and mix thoroughly. Buffer A1 with RNase should be stored at 4 °C for frequent use and at -20 °C for infrequent use. Add 32 mL (MB21001) or 60 mL (MB21002) of 100% molecular biology grade ethanol to Buffer AS. Buffer A2 may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37 °C. Buffer A3 contains guanidine hydrochloride. Wear gloves and goggles when using this kit.

System Components

Component	50 columns	200 columns
Buffer A1	15 mL	60 mL
Buffer A2	15 mL	60 mL
Buffer A3	20 mL	80 mL
Buffer AS (concentrate)	8 mL	2 × 15 mL
Buffer AE (does not contain EDTA)	15 mL	60 mL
RNase A	5 mg	25 mg
NZYTech Spin Columns	50	200
Collection Tubes (2 mL)	50	200

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 vigorous shaking.

Protocol for plasmid DNA purification from *Escherichia coli* cells

All centrifugations should be carried out at room temperature in a table-top microcentrifuge at >12000 x*g* (10000-15000 rpm depending on the rotor type).

1. Cultivate and harvest bacterial cells

Pellet 1-5 mL of an *E. coli* LB culture for 30 s. Discard supernatant. Remove as much media as possible.

2. Cell lysis

Re-suspend cell pellet completely in 250 µL of Buffer A1 by vigorous vortexing/pipetting.

Add 250 µL of Buffer A2 and mix gently by inverting the tube for 5 times. Incubate at room temperature (10-25 ºC) for up to 2 min. Do not vortex.

Add 300 µL of Buffer A3. Mix gently by inverting the tube for 6-8 times. Do not vortex.

3. Clarification of lysate

Centrifuge for 3 min at room temperature to pellet precipitate.

4. Bind DNA

Place NZYTech spin column in a 2 mL collecting tube and load the supernatant from step 3 onto the column. Centrifuge for 30 s. Discard flow-through.

5. Wash silica membrane

Add 600 µL of Buffer AS (make sure ethanol was previously added). Centrifuge for 1 min. Discard flow-through.

6. Dry silica membrane

Re-insert the NZYTech spin column into the empty 2 mL collecting tube and centrifuge for 1 min.

7. Elute highly pure DNA

Place the dried NZYTech spin column into a clean 1.5 mL microcentrifuge tube and add 50 µL of Buffer AE. Incubate for 1 min at room temperature (10-25 ºC). Centrifuge for 1 min. By repeating this step the overall yield will increase by 15-20%. To obtain a highly concentrated miniprep (1.3 times higher) reduce the volume of elution buffer to 30 µL. Store the purified DNA at -20 °C.

Note: It is extremely important to add the Elution Buffer into the centre part of the column. Incubating the column with the Elution Buffer at higher temperatures (37 to 50 °C) may slightly increase the yield especially of large (>10,000 bp) DNA Plasmids. Pre-warming the Elution Buffer at 55 to 80 °C may also slightly increase elution efficiency. If water is used for elution, make sure that its pH is between 7.0 and 8.5. Elution efficiency is dependent on pH and the maximum elution efficiency is achieved within this range. A pH <7.0 can decrease yield.

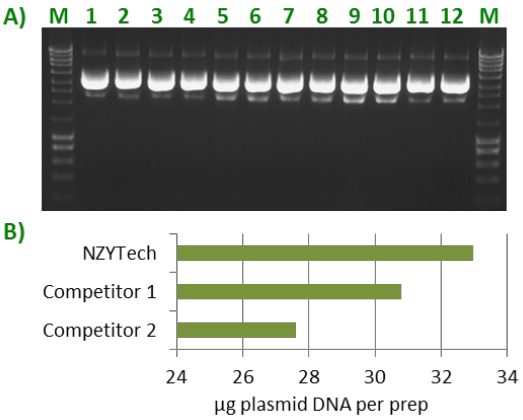

Figure 1. Plasmid DNA was isolated from 3 mL of a recombinant *Escherichia coli* strain using three different rapid protocols (NZYTech, lanes 1-4; Competitor 1, lanes 5-8; Competitor 2, lanes 9-12). DNA isolation was performed according to each manufacturer´s protocol. The quadruplicates (2 µL of 50 µL eluate) were

analysed through agarose gel electrophoresis (A). Eluates were also analysed by UV/VIS spectroscopy to determine total yields (B).

Quality control assay

All components of NZYSpeedy Miniprep kit are tested following the isolation protocol described above. The purification system must isolate 15-35 µg of pNZY28 plasmid DNA per column.

V2101

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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