NZYMiniprep 96 well plate

Catalogue numbers:

MB20201, 2 plates
MB20202, 4 x 2 plates

Description

NZYMiniprep 96 well plate kit is designed for the manual or automated rapid, small-scale preparation of highly pure plasmid DNA from recombinant *Escherichia coli* strains. The method is based on the alkaline lysis of bacterial cells followed by adsorption of DNA onto silica in the presence of high salt. The plasmid DNA is selectively adsorbed in the silica gel-based NZYMiniprep 96 well plate 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. NZYMiniprep 96 well plate kit includes an additional washing buffer (AY) which is strongly recommended for the complete removal of high levels of endonucleases. NZYMiniprep 96 well plate kit allows simultaneous processing of up to 96 samples, typically in less than 45 min. Actual processing times may depend on the configuration of the liquid handling system used. Typically yields of 5–15 µg plasmid DNA can be purified from 1.5 mL overnight cultures. The DNA binding capacity is about 20 µg/well. The final concentration of the eluted DNA is 50–200 ng/µL (depending on the elution buffer volume and the bacterial culture). The kit is designed for use with vacuum manifolds or under centrifugation.

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 60 mL of 100% molecular biology grade ethanol to each bottle of buffer A4. Buffer A2 may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37 °C. Buffers A3 and AY contain guanidine hydrochloride. Wear gloves and goggles when using this kit.

System Components

Component	2 x 96 preps
Buffer A1	60 mL
Buffer A2	60 mL
Buffer A3	80 mL
Buffer AY	120 mL
Buffer A4 (concentrate)	5 x 15 mL
Buffer AE (does not contain EDTA)	60 mL
RNase A (lyophilised)	25 mg
NZY Plasmid filter plate (violet rings)	2
NZY Plasmid binding plate (white rings)	2
Elution plate (V-bottom)	2
96-deep-well block* (1.2 mL)	4

* For centrifugation processing only.

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. It is recommended the use 24 or 96-deep well plates for growing bacteria. Do not exceed the volume of 1.5 mL when using 96-deep well plates. Cover the deep well plates with a gas-permeable foil. Incubate cells for 12–16 h at 37 °C with vigorous shaking.

Protocol for plasmid DNA purification from *Escherichia coli* cells

This kit was developed to use under vacuum, which requires a vacuum manifold, or under centrifugation. NZYMiniprep 96 well plate kit provides accessory plates (e.g. 96-deep-well blocks or elution plates). According to the equipment available, proceed with the vacuum (see Section A) or centrifuge procedure (see Section B).

A. Vacuum procedure

All purification steps should be carried out at **room temperature**.

1. Harvesting of bacterial cells

Centrifuge the bacteria cultures for 10 min at 1,000 x *g*. Discard supernatant. Remove as much media as possible by very gently laying plates upside down on a clean soft tissue.

2. Cell lysis

Re-suspend cell pellets in 250 µL Buffer A1 **with RNase A** by vigorous vortexing.

Add 250 µL of Buffer A2 and mix gently at room temperature for a maximum of 4 min. Check for the formation of a clear viscous lysate. Do not vortex.

Add 350 µL Buffer A3 and mix gently at room temperature. Check for the formation of a precipitate. Do not vortex.

3. Assemble the Vacuum manifold following the manufacturer recommendations

Place the NZY Plasmid Binding Plate (white rings) in the adjusted below position to receive the flow through of NZY Plasmid Filter Plates (violet rings) located on top.

4. Transfer crude lysates to NZY Plasmid Filter Plate

Transfer the entire lysate volume (approximately 850 µL) into the wells of NZY Plasmid Filter Plate (violet rings). Column wise processing is recommended.
Note: In case of lower than 96 number of samples are to be processed cover the non-used wells with a self-adhering plastic foil to maintain sufficient vacuum.

5. Collect clear crude lysate in NZY Plasmid Binding Plate

Apply vacuum (-0.2 to -0.4 bar, 1-5 min) to allow clear crude lysates to pass through the filter plate such that they will be collected in the NZY Plasmid Binding Plate (white rings).
Note: Check if all samples have passed through NZY Plasmid Filter Plate (violet rings) before proceeding to next step.

6. Reassemble the vacuum manifold

Remove and discard the NZY Plasmid Filter Plate (violet rings). Transfer the NZY Plasmid Binding plate (white rings) filled with the clear crude lysates into the top vacuum position.

7. Bind DNA to the plate silica membranes

Apply vacuum (-0.2 to -0.4 bar, 1 min) to allow clear crude samples to pass through the NZY Plasmid Binding Plate while DNA binds to the silica membrane.
Note: Check if all samples have passed through the columns before proceeding to next step.

8. Wash silica membranes

Add 500 µL of Buffer AY to the NZY Plasmid Binding Plate. Apply vacuum (-0.2 to -0.4 bar, 1 min) to allow washing the DNA bound to the silica membrane.
Note: Check if all Buffer AY has passed through the columns before proceeding to next step.

Add 900 µL of A4 Buffer (with ethanol added) to the NZY Plasmid Binding Plate. Apply vacuum (-0.2 to -0.4 bar, 1 min) to allow washing of the DNA bound to the silica membrane.
Note: Check if all Wash Buffer has passed through the columns before proceeding to next step.

Repeat the washing step with 900 µL of A4 buffer.

9. Dry silica membrane

Remove any residual washing buffer from the NZY Plasmid Binding Plate by gently tapping the outlets of the plate onto a soft tissue until no drops come out. Re-assemble the plate into the vacuum manifold and apply vacuum (-0.4 to -0.6 bar) for at least 10-15 min to dry the silica membrane completely. This step is absolutely required to remove traces of ethanol, which inhibits enzymatic reactions.

10. Elute highly pure DNA

After finishing the drying step insert the elution plate with V-bottom into the manifold to recover purified DNA. Add 75-150 µL of Elution Buffer (125 µL is the recommended volume, see Elution efficiencies section) or highly pure water (pH 8.5) to each well of the NZY Plasmid Binding Plate. Incubate for 1-3 min at room temperature (optionally), apply vacuum (-0.4 to -0.6 bar) for 1-2 min and collect the eluted DNA. Remove the elution plate from vacuum manifold and seal it with the supplied adhesive cover foil for further storage.

Notes:
- The Elution Buffer should be dispensed onto the centre of the silica membrane.

- Eluting the purified DNA in a centrifuge may be necessary when the purified DNA is required at higher concentrations. Using a centrifuge allows the dispensing volume to be reduced down to 50-75 µL. Centrifuge for 2-4 min in a microtiter plate centrifuge at 5,600 x g to collect the DNA.

B. Centrifuge procedure

All purification steps should be carried out at **room temperature**.
All centrifugations should be carried out at **room temperature** in a microtiter plate centrifuge which reaches accelerations of 5,600-6,000 x*g*. It is recommended to perform 2 ×96 DNA preparations at one time to allow that the rotor is balanced.

1. Harvesting of bacterial cells

Centrifuge the bacteria cultures for 10 min at 1,000 x *g*. Discard supernatant. Remove as much media as possible by very gently laying plates upside down on a clean soft tissue.

2. Cell lysis

Re-suspend cell pellets in 250 µL Buffer A1 **with RNase A** by vigorous vortexing.
Add 250 µL of Buffer A2 and mix gently at room temperature for a maximum of 4 min. Check for the formation of a clear viscous lysate. Do not vortex.
Add 350 µL Buffer A3 and mix gently at room temperature. Check for the formation of a precipitate. Do not vortex.

3. Transfer crude lysates to NZY Plasmid Filter Plate

Place NZY Plasmid Filter Plate (violet rings) on top of a new 96-deep-well block. Transfer the entire lysate volume (approximately 850 µL) into the wells of the NZY Plasmid Filter Plate (violet rings). Column wise processing is recommended.
Note: In case of lower than 96 number of samples are to be processed cover the non-used wells with a self-adhering plastic foil to maintain sufficient vacuum.

4. Clear crude lysate using centrifugation

Place NZY Plasmid Filter Plate (violet rings) with 96-deep-well block onto the centrifugation carrier and centrifuge at 5,600 ×*g* for 4 min. **Save the flow-through**.

5. Bind DNA to the plate silica membranes

Transfer the flow-through into the wells of NZY Plasmid Binding Plate (white rings) which was previously placed on top of new 96-deep-well block. Load NZY Plasmid Binding Plate (white rings) with 96-deep-well block onto the centrifugation carrier. Centrifuge at 5,600 ×*g* for 4 min to allow clear crude samples to pass through the NZY Plasmid Binding Plate (white rings) while DNA binds to the silica membrane.
Note: Check if all samples have passed through the columns before proceeding to next step.

6. Wash silica membranes

Discard the flow-through and place the NZY Plasmid Binding Plate (white rings) back into the 96-deep-well block.

Add 500 µL of Buffer AY to each well of the NZY Plasmid Binding Plate. Centrifuge at 5,600 ×*g* for 4 min to allow washing the DNA bound to the silica membrane. Discard the flow-through and place the NZY Plasmid Binding Plate back into the 96-deep-well block.
Note: *Check if all Buffer AY has passed through the columns before proceeding to next step.*

Add 900 µL of A4 Buffer (with ethanol added) to the NZY Plasmid Binding Plate. Centrifuge at 5,600 ×*g* for 4 min to allow washing of the DNA bound to the silica membrane. Discard the flow-through and place the NZY Plasmid Binding Plate back into the 96-deep-well block.
Note: *Check if all Wash Buffer has passed through the columns before proceeding to next step.*

Repeat the washing step with 900 µL of A4 buffer. Centrifuge again at 5,600 ×*g* for 4 min.

7. Dry silica membrane

Discard the flow-through from the 96-deep-well block and remove any residual washing buffer from the NZY Plasmid Binding Plate by gently tapping the outlets of the plate onto a soft tissue until no drops come out. Re-assemble the plate into the 96-deep-well block. Centrifuge at 5,600 ×*g* for at least 10-15 min to dry the silica membrane completely. This step is absolutely required to remove traces of ethanol, which inhibits enzymatic reactions.

8. Elute highly pure DNA

After finishing the drying step place the NZY Plasmid Binding Plate on new elution plate with V-bottom. Add 75-150 µL of Elution Buffer (125 µL is the recommended volume, see Elution efficiencies section) or highly pure water (pH 8.5) to each well of the plate. Incubate for 1-3 min at room temperature (optionally). Centrifuge at 5,600 ×*g* for 4 min and collect the eluted DNA. Remove the elution plate and seal it with the supplied adhesive cover foil for further storage.
Note: The Elution Buffer should be dispensed onto the centre of the silica membrane.

Elution efficiencies

The efficiency of the DNA elution depends on the pH of the elution buffer. Elution is most effective at pH 8.0–8.5. When using nuclease-free water for elution, the pH value should be checked and, if necessary, adjusted to 8.0–8.5. Lower pH of the selected elution buffer may lead to lower recoveries. Yield of larger DNA fragments (> 5–10 kbp) can be increased by using pre-warmed (70 °C) elution buffer. An elution volume of 75–150 µL (125 µL preferably) of Elution Buffer, as well as a 3–5 min incubation at room temperature (18–25 °C) of the elution buffer on the silica membrane are recommended. When using a vacuum manifold, the recovery elution volumes vary from 35 to 110 µL, when the volumes of dispensed Elution Buffer vary from 75 to 150 µL, respectively. Typical recoveries of total plasmid DNA range between 75 to 95% of the initial DNA.

Quality control assay

All components of NZYMiniprep 96 well plate kit are tested following the isolation protocol described above. The purification system must isolate 15-45 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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