NeoPrep mini

Catalogue number	Presentation
NB-60-0001	50 columns

Description

NeoPrep mini kits are designed for the rapid, small-scale preparation of highly pure plasmid DNA from recombinant Escherichia coli strains. Neo Biotech´s miniprep procedure 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 Neo Biotech 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. NeoPrep mini kit includes an additional washing buffer (AY) which is strongly recommended for the complete removal of high levels of endonucleases. To isolate DNA from low copy number plasmids, BACs or cosmids, or to obtain higher DNA concentrations, use 10 mL of E. coli cultures and double the volumes of Buffers A1, A2 and A3.

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	NB-60-0001 (50 COLUMNS)
Buffer A1	15 mL
Buffer A2	15 mL
Buffer A3	20 mL
Buffer AY	30 mL
Buffer A4 (concentrate)	8 mL
Buffer AE (does not contain EDTA)	15 mL
RNase A	5 mg
Neo Biotech Spin Columns	50
Collection Tubes (2 mL)	50

Reagents, Materials and Equipment Required but Not Provided

- 96-100% ethanol
- 1,5 mL microcentrifuge tubes and disposable tips
- Centrifuge for 1,5 mL microcentrifuge tubes

Standard Protocol

Recommendations before starting

- 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.

Procedures before starting

Reagents Preparation

- Buffer A1: 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 A should be stored at 4 °C for frequent use and at -20 °C for infrequent use.
- Buffer A4: add 32 mL of 96-100% ethanol to the bottle of Buffer A4.

Procedure

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

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

Pellet 1-5 mL of an E. coli LB culture for 30 s. Discard supernatant. Remove as much media as possible. For low copy number plasmids double the volume of cells and of lysis Buffers A1, A2 and A3.

2. Cell Lysis

Re-suspend cell pellet in 250 µL Buffer A1 by vigorous vortexing.

Add 250 µL of Buffer A2 and mix gently by inverting the tube for 6-8 times. Incubate at room temperature for a maximum of 4 min. Do not vortex.

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

3. Lysate Clarification

Centrifuge for 5-10 min at room temperature, depending on initial culture volume.

4. DNA Binding

Place Neo Biotech spin column in a 2 mL collecting tube and load the supernatant from step 3 onto the column. Centrifuge for 1 min at 11,000 xg. Discard flow-through.

5. Wash Silica Membrane

Add 500 µL of Buffer AY onto the column. Centrifuge for 1 min. Discard flow-through. This step is crucial to increase the reading length of DNA sequencing reactions and to improve the performance of critical enzymatic reactions. When using endA+ strains, such as JM series, HB101 and its derivatives, or any wild-type strain, use pre-warmed Buffer AY (50 °C).

Add 600 µL of Buffer A4 (make sure ethanol was previously added). Centrifuge for 1 min. Discard flow-through.

6. Dry Silica Membrane

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

7. Elute Highly Pure DNA

Place the dried Neo Biotech spin column into a clean 1.5 mL microcentrifuge tube and add 50 µL of Buffer AE. Incubate 1 min at room temperature. 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.

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 vigorous shaking.

Quality control assay

All components of NeoPrep mini kit are tested following the isolation protocol described above. The purification system must isolate 15-45 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.
• **Inadequate Buffers preparation**
Check that Buffer A4 concentrated was diluted with correct volume of ethanol.
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 A1. See Reagents preparation described above.
• **Degraded DNA/DNase contamination**
Check your working area and pipettes for possible DNAse contamination. Implement stringent cleanliness protocols.

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 NeoPrep mini kit.

For life science research only. Not for use in diagnostic procedures.