NeoPrep mini

NB-60-0001

NeoPrep mini

#Cat: NB-60-0001 **Size: 50 colums**

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 NeoPrep mini 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. NEO BIOTECH NeoPrep mini kit includes an additional washing buffer (AY) whichis 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 mLof E. coli cultures and double the volumes of Buffers A1, A2 and A3.

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 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

- 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

Growing of bacterial cultures

LB medium is recommended for cultivation of bacterial cells. Alternatively, rich media like 2xYT 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.

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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 xg (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. 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. Clarification of lysate

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

4. Bind DNA

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 NeoPrep mini (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.

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Data:

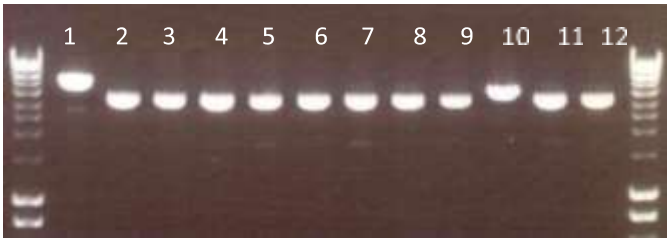

Figure 1. Agarose gel electrophoresis of pNZY28 derivatives (2 µL) digested with EcoR I (lanes 1 to 12).

Quality control assay

All components of Neo Biotech NeoPrep mini kit are tested following the isolation protocol describedabove. The purification system must isolate 15-45 µg of pNZY28 plasmid DNA per column.