EasyPrep High Pure Plasmid Mini Plus Extraction kit

For purification of molecular biology grade DNA

Contents

Introduction

EasyPrep High Pure Plasmid Mini Plus Extraction kit is based on alkaline lysis technology followed by DNA adsorption onto silica membrane in the presence of a high salt concentration. Plasmid DNA purified using this product is ready for immediate use. Phenol extraction and ethanol precipitation are not required. High-quality plasmid DNA is eluted in a small volume of Tris Buffer or deionized water. This protocol is designed for purification of up to 70 μg of plasmid DNA from 5–15 mL of overnight culture of *E. coli* grown in Luria–Bertani (LB) medium.

Plasmid DNA prepared using EasyPrep High Pure Plasmid Mini plus kit is suitable for a variety of routine applications, including restriction enzyme digestion, sequencing, library screening, ligation and transformation, in vitro translation, and transfection of robust cells.

Yields

Plasmid Type	Bacterial Cells Volume	Plasmid Yield	Plasmid
Low Copy	5-15 ml	5-25 μg	pBR322, pACYC, pSC101, sUPERcOS, pWE15
High Copy	5-15 ml	15-70 μg	pTZ, pUC, pBS, pGM-T

Key notes

1. Add the provided RNase A solution to Buffer PB1, mix, and store at 2°C–8°C.
2. Add ethanol (96%–100%) to Buffer PBW before use (refer to the bottle label for volume). For example, add 60 ml ethanol (96%–100%) to 15 mL of Buffer PBW or add 200 mL of ethanol (96%–100%) to 50 mL of Buffer PBW.
3. Check Buffers BBL, PB2, and PB3 for salt precipitation before use. If found, redissolve the precipitates by warming the buffers to 37°C. Do not shake Buffer PB2 vigorously.
4. Avoid direct contact with buffers PB2 and P4. Close the bottles containing buffer PB2 and P4 immediately after use to avoid acidification of buffers due to CO_2 present in air.
5. Perform all centrifugation steps at 12,000 rpm (~13,400 ×g) in a table-top microcentrifuge at room temperature.
6. Use the Spin Column CP4 soon after treatment with Buffer BBL because long-term placement may affect the purifying effect.
7. Use Buffer PBD to efficiently remove the residual protein. This step is essential when working with endA + strains, such as the JM series, HB101 and its derivatives, or any wild-type strain, to ensure that plasmid DNA has not been degraded.

Kit Contents

Contents	DPT-BA07 (50 preps)
RNase A (10 mg/mL)	300 µl
Buffer BBL	30 ml
Buffer PB1	30 ml
Buffer PB2	30 ml
Buffer PB3	40 ml
Buffer PBD	30 ml
Buffer PBW	15 ml
Buffer TB	15 ml
Filtration Columns CS	50
Spin Columns CP4	50
Collection Tubes (2 mL)	100

Storage

EasyPrep High Pure Plasmid Mini Plus Extraction kit can be stored dry at room temperature (15°C–25°C) for up to 12 months with no reduction in performance and quality. Any precipitate formed in buffers after storage at 2°C–8°C should be dissolved by warming the buffers to 37°C before use. RNaseA (10 mg/mL) can be stored for one year at room temperature. After the addition of RNaseA, Buffer PB1 remains stable for 6 months at 2°C–8°C.

Protocol

1. Column equilibration: Add 500 µL of Buffer BBL to the Spin Column CP4. Centrifuge for 1 min at 12,000 rpm (~13,400 ×g) in a table-top microcentrifuge. Discard the flow-throw and place the Spin Column CP4 into the collection tube.
2. Harvest 5–15 mL of bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400 ×g) in a conventional, table-top microcentrifuge for 1 min at room temperature (15°C–25°C), and then remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.
3. Resuspend pelleted bacterial cells in 500 µL of Buffer PB1.

Note: Ensure that RNase A has been added to Buffer PB1. No cell clumps should be visible after resuspension of the pellet.

4. Add 500 µL of Buffer PB2 and mix thoroughly by inverting the tube 6–8 times.

Note: Mix gently by inverting the tube. Do not vortex because this will result in shearing of the genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

5. Add 700 µL of Buffer PB3 and mix immediately and thoroughly by inverting the tube 6–8 times. The solution should appear cloudy.

Note: Mix the solution thoroughly immediately after the addition of Buffer PB3 to avoid localized precipitation.

6. Centrifuge for 10 min at 12,000 rpm (~13,400 ×g) in a table-top microcentrifuge. **(Extend the centrifugation time if too much liquid is present in the column).** Collect as much supernatant as possible if a compact, white pellet forms.
7. Apply supernatants from step 6 to the Filtration Column CS (place the CS in a collection tube) by decanting or pipetting. Centrifuge for 2 min at 12,000 rpm (~13,400 ×g) in a table-top microcentrifuge.
8. Transfer the flow-through to the Spin Column CP4 (place the CP4 in a collection tube). Centrifuge for 1 min at 12,000 rpm (~13,400 ×g). Discard the flow-through.
9. Wash the Spin Column CP4 by adding 500 uL of Buffer PBD and centrifuging for 1 min at 12,000 rpm (~13,400 ×g). Discard the flow-through.
10. Wash the Spin Column CP4 by adding 700 µL of Buffer PBW (ensure that 96%–100% ethanol has been added to Buffer PBW) and centrifuging for 1 min at 12,000 rpm (~13,400 ×g). Discard the flow-through.
11. Wash the Spin Column CP4 by adding 500 µL of Buffer PBW and centrifuging for 1 min at 12,000 rpm (~13,400 ×g).
12. Discard the flow-through and centrifuge for an additional 2 min at 12,000 rpm (~13,400 ×g) to remove residual wash buffer PBW.

Note: Residual wash buffer will not be completely removed unless the flow-through has been discarded before this additional centrifugation. We suggest opening the CP4 lid and keeping it at room temperature

for a while. Residual ethanol from Buffer PBW may inhibit subsequent enzymatic reactions.

13. Place the Spin Column CP4 in a clean, 1.5-mL microcentrifuge tube. To elute DNA, add 100–300 μL of Buffer TB or water (pH 7.0–8.5) to the center of each Spin Column CP4, let it stand for 2 min, and centrifuge for 1 min at 12,000 rpm (~13,400 ×g).

Note: Repeat step 13 to increase plasmid callback efficiency. If the volume of eluted buffer is less than 1 mL, the recovery efficiency may be affected. The pH value of the eluted buffer will have some influence in eluting; Buffer TB or distilled water (pH 7.0–8.5) is indicated for eluting the plasmid DNA. Eluting in Buffer TB and storage at –20°C is recommended for long-term storage of DNA because the stored DNA in water is susceptible to acid hydrolysis.