EasyPrep HY-Midi Plasmid Extraction Kit

For the purification of highly pure plasmid DNA with a high yield

Contents

Introduction

The EasyPrep HY-Midi Plasmid Extraction Kit contains a specially designed buffer system. This system is used in conjunction with isopropanol precipitation to achieve fast and efficient purification of highly pure plasmid DNA. The plasmid DNA prepared using this kit is suitable for various routine applications, such as restriction enzyme digestion, sequencing, library screening, ligation and transformation, in vitro translation, and transfection of cell lines. For high-copy vectors, a plasmid volume of 500–1500 μg and bacterial culture volume of 100 mL are recommended. For low-copy vectors, a plasmid volume of 200–400 μg and bacterial culture volume of 200 mL are recommended.

Using the EasyRed reagent

The EasyRed reagent is a color indicator that provides visual identification of optimal buffer mixing. This reagent prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of sodium dodecyl sulfate (SDS), genomic DNA, and cell debris. Researchers can determine whether to use EasyRed according to their experience and research purpose. EasyRed has no influence on reactions such as polymerase chain reaction (PCR), enzyme digestion, and sequencing. However, if purified plasmids must be used in a transfection experiment, the usage of EasyRed is not recommended. EasyRed should be added to Buffer PB1 at a ratio of 1:200 before the buffer is used; then, the product should be mixed thoroughly by inverting it, and the obtained solution should be transparent red.

The plasmid preparation procedure is performed as usual. After adding Buffer PB1 to harvested bacterial cells, the suspension becomes cloudy. After adding Buffer PB2 to Buffer PB1, the color of the suspension changes to purple. Mixing should result in a homogeneously transparent purple suspension, which indicates that lysis is complete. Adding Buffer PB4 results in EasyRed turning transparent yellow. The presence of a homogeneous solution with no traces of purple indicates that SDS has been effectively precipitated from the lysis buffer (Buffer PB2).

Important notes

1. Prevent direct contact between Buffer PB2 and Buffer PB4. Close the bottle containing Buffer PB2 and Buffer PB4 immediately after use to avoid the acidification of the buffers due to the CO_2 in the air.
2. Draw out the plunger from the filter slowly to avoid loosening the membrane.
3. The amount of extracted plasmid is related to the cell concentration and plasmid copy. When working with low-copy vectors or large plasmids (>10 kb), increasing the culture volume in proportion to Buffer PB1, Buffer PB2, and Buffer PB4 may be beneficial. Heat Buffer TB to 50–60 °C before use. Extend the adsorption and elution time as necessary to increase extraction efficiency.

Preliminary matters

1. Add the provided RNase A solution (500 μL) to Buffer PB1, mix, and store at 2–8 °C.

2. Check Buffer PB2 and Buffer PB4 for SDS precipitation due to low storage temperatures. Redissolve the precipitate by heating the mixture to 37 °C. Do not shake Buffer PB2 vigorously.
3. Prepare 60 mL of 5 M NaCl solution.
4. Optional: Add the provided EasyRed reagent to Buffer PB1 at a ratio of 1:200 before use. However, if the purified plasmids must be used in the transfection experiment, the use of EasyRed is not recommended.

Kit Contents

Contents	DPT-BA16 (10 preps)
RNase A (100 mg/mL)	500 µL
Buffer PB1	100 mL
Buffer PB2	100 mL
Buffer PB4	100 mL
Buffer TB	30 mL
EasyRed	500 µL
Filtration Column CS1	10

Storage

The EasyPrep HY-Midi Plasmid Extraction Kit can be stored in dry conditions at room temperature (15–25 °C) for up to 12 months without degradation of performance or quality. RNase A (100 mg/mL) can be stored for 1 year at room temperature (1–25 °C) without any reduction in quality. After RNase A is added to Buffer PB1, the buffer remains stable for 6 months when stored at 2–8 °C.

Protocol

1. Harvest 100 mL of bacterial culture cells by centrifuging at 10,000 rpm (~11,500 × g) for 3 min at room temperature (15–25 °C). Then, remove all traces of the supernatant by inverting the open centrifuge tube until the medium has been completely drained.
2. Resuspend pelleted bacterial cells in 10 mL of Buffer PB1.

Note: Ensure that RNase A has been added to Buffer PB1. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain. If EasyRed reagent has been added to Buffer PB1, mix thoroughly before use. The suspension becomes cloudy red due to the presence of bacteria cells.

3. Add 10 mL of Buffer PB2 to the suspension, mix thoroughly by inverting the tube 6–8 times, and incubate the mixture at room temperature for 5 min.

Note: Mix by inverting the tube. Do not vortex because vortexing results in the shearing of 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.

If EasyRed is added to Buffer PB1, the cell suspension turns purple after the addition of Buffer PB2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized red regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

4. Add 10 mL of Buffer PB4 to the suspension and mix immediately and thoroughly by vigorously inverting the tube 6–8 times until the solution becomes cloudy. Incubate the solution at room temperature for 10 min. A white fluffy material forms.

Note: To avoid localized precipitation, mix the lysate thoroughly and immediately after the addition of Buffer PB4. Perform mixing by inverting the tube, and do not vortex.

If EasyRed is used, the suspension should be mixed until all traces of purple are eliminated and the suspension becomes transparent yellow. A homogeneous yellow suspension indicates that SDS has been effectively precipitated.

5. Centrifuge the suspension for 10 min at 10,000 rpm (~11,500 × g). The white material should be precipitated to the bottom of the centrifuge tube and form a white pellet.

Note: The centrifugation time can be increased appropriately to precipitate the white material. If more than 100 mL of bacterial culture is used, the centrifugation time can be increased to 20–30 min.

6. Transfer the supernatant into Filtration Column CS1. Gently insert the plunger into the filtration column and filter the cell lysate into a new 50-mL tube (not supplied in the kit).

Note: If the white material does not precipitate to the bottom in step 5, it indicates that the lysate was not mixed thoroughly in step 5. In this scenario, avoid transferring large cell clumps into Filtration Column CS1. These clumps will clog the filtration membrane. Small fragments have no influence on Filtration Column CS1.

7. Add isopropanol in 50% volume of cleared lysate. Then, add 5M NaCl of half amount of the isopropanol to the mixture. Seal the received tube and mix thoroughly by inverting the tube.

Note: Visible precipitate should generally not appear in this step.

Optional: Higher plasmid yields can be obtained after a 30 min incubation at -20°C.

8. Centrifuge the mixture at 10,000 rpm (~11,500 × g) for 30 min at 4 °C, and decant all the supernatant.
9. Wash the pellet with 6 mL of 70% ethanol. Centrifuge the mixture at 10,000 rpm (~11,500 × g) for 10 min at 4 °C. Decant all the supernatant without disturbing the pellet.
10. Repeat step 9.
11. Air-dry the plasmid DNA pellet for 10–20 min, and redissolve the DNA in 1–1.5 mL of Buffer TB.

Note: Overdrying the pellet makes it difficult to redissolve the DNA. DNA dissolves best under slightly alkaline conditions. Buffer TB or distilled water (pH 7.0–8.5) is recommended to be used for redissolving the plasmid DNA. DNA can be dissolved in Buffer TB and stored at −20 °C to avoid possible acid hydrolysis in water during long-term storage.