PureYield™ Plasmid Miniprep System

INSTRUCTIONS FOR USE OF PRODUCTS A1220, A1221, A1222 AND A1223

Quick PROTOCOL

Solution Preparation

Before lysing cells and purifying DNA, prepare the Column Wash Solution by adding ethanol. Cap tightly after addition. See Technical Bulletin #TB374 for detailed instructions.

DNA Purification by Centrifugation

Prepare Lysate

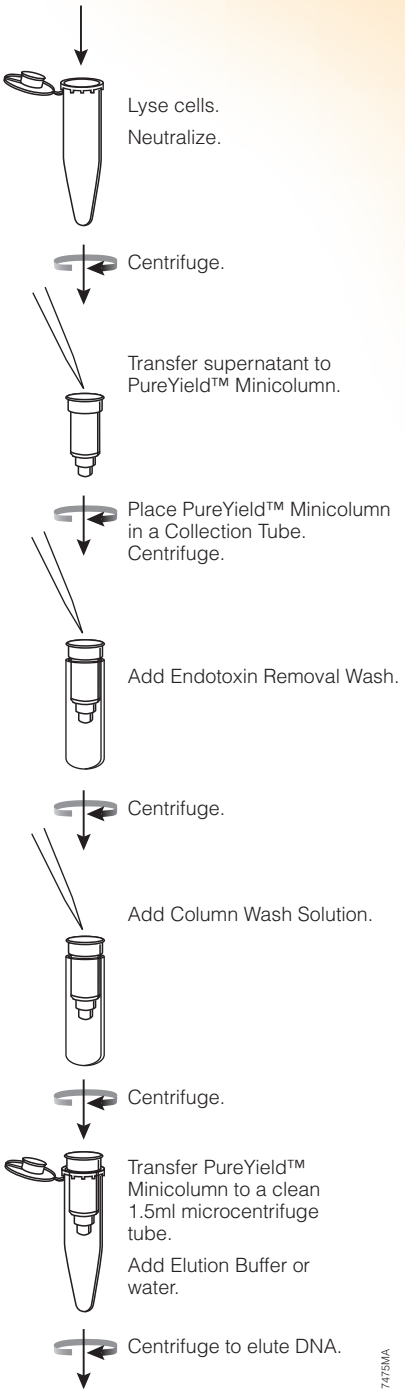
1. Add 600µl of bacterial culture to a 1.5ml microcentrifuge tube.
   Note: For higher yields and purity use the alternative protocol below to harvest and process up to 3ml of bacterial culture.
2. Add 100µl of Cell Lysis Buffer (Blue), and mix by inverting the tube 6 times.
3. Add 350µl of cold (4–8°C) Neutralization Solution, and mix thoroughly by inverting.
4. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.
5. Transfer the supernatant (~900µl) to a PureYield™ Minicolumn without disturbing the cell debris pellet.
6. Place the minicolumn into a Collection Tube, and centrifuge at maximum speed in a microcentrifuge for 15 seconds.
7. Discard the flowthrough, and place the minicolumn into the same Collection Tube.

Wash

8. Add 200µl of Endotoxin Removal Wash (ERB) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 15 seconds.
9. Add 400µl of Column Wash Solution (CWC) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 30 seconds.

Elute

10. Transfer the minicolumn to a clean 1.5ml microcentrifuge tube, then add 30µl of Elution Buffer or nuclease-free water directly to the minicolumn matrix. Let stand for 1 minute at room temperature.
11. Centrifuge for 15 seconds to elute the plasmid DNA. Cap the microcentrifuge tube, and store eluted plasmid DNA at –20°C.



Alternative Protocol for Larger Culture Volumes

1. Centrifuge 1.5ml of bacterial culture for 30 seconds at maximum speed in a microcentrifuge. Discard the supernatant.
2. Add an additional 1.5ml of bacterial culture to the same tube and repeat Step 1.
3. Add 600µl of TE buffer or water to the cell pellet, and resuspend completely.
4. Proceed to Step 2 of the standard protocol above.

For complete protocol information see Technical Bulletin #TB374, available at: **www.promega.com/tbs**

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PureYield™ Plasmid Miniprep System

INSTRUCTIONS FOR USE OF PRODUCTS A1220, A1221, A1222 AND A1223

Quick PROTOCOL

DNA Purification by Vacuum

Prepare Lysate

1. Transfer 1.5ml of culture to a 1.5ml microcentrifuge tube
   Note: If you wish to process larger volumes of bacterial culture (up to 3ml) use the alternative protocol provided below.
2. Centrifuge at maximum speed in a microcentrifuge for 1 minute.
3. Remove and discard medium.
4. Resuspend the cell pellet in 600µl of TE buffer or water.
5. Add 100µl of Cell Lysis Buffer (Blue), and mix by inverting the tube 6 times.
6. Add 350µl of cold (4–8°C) Neutralization Solution, and mix thoroughly by inverting.
7. Centrifuge at maximum speed in a microcentrifuge for 3 minutes. Place a PureYield™ minicolumn on a Luer-Lok® adapter of a VacMan® or VacMan® Jr Laboratory Vacuum manifold
8. Transfer the supernatant (~900µl) into a PureYield™ Minicolumn.
9. Apply vacuum pulling the lysate through the column.

Wash

10. Add 200µl of Endotoxin Removal Wash (ERB) to the minicolumn. Allow the vacuum to pull the solution through the column.
11. Add 400µl of Column Wash Solution (CWC) to the minicolumn. Allow the vacuum to pull the solution through the column. Release the vacuum, and remove the PureYield™ Minicolumn.

Elute

12. Place the column in a 2ml collection tube, and centrifuge at maximum speed in a microcentrifuge for 1 minute.
13. Transfer the minicolumn into a clean 1.5ml microcentrifuge tube, then add 30µl of Elution Buffer or nuclease-free water directly to the minicolumn matrix. Let stand for 1 minute at room temperature.
14. Centrifuge for 15 seconds to elute the plasmid DNA. Cap the microcentrifuge tube, and store eluted plasmid DNA at –20°C.

For complete protocol information see Technical Bulletin #TB374, available at: www.promega.com/tbs

Alternative Protocol for Larger Culture Volumes

1. Centrifuge 1.5ml of bacterial culture for 30 seconds at maximum speed in a microcentrifuge.
2. Discard the supernatant.
3. Add an additional 1.5ml of bacterial culture to the same tube. Repeat Steps 1 and 2.
4. Add 600µl of TE buffer or water to the cell pellet, and resuspend completely.
5. Proceed to Step 5 of the standard protocol above.

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