

PRODUCT INFORMATION

Cyclo-Prep™ (K179)

- Fast Spin Column Format for Efficient Sample Preparation
- Phenol-Free Procedure Reduces Laboratory Hazards
- Ideal for Automated Fluorescent Sequencing

Cyclo-Prep is a spin column-based kit for rapid purification of plasmid DNA from 1-2 ml liquid culture. In just 15 minutes, prepare ultra-pure plasmid DNA ($A_{260}/A_{280} = 1.8$) at a yield of 2-15 µg per ml *E. coli* culture using your tabletop microcentrifuge.¹ The DNA isolated from Cyclo-Prep is suitable for automated fluorescent sequencing², PCR, *in vitro* transcription, transformation and restriction enzyme digestion. The simple protocol provides an environmentally safe and convenient alternative to common phenol-based DNA purification procedures.

The Cyclo-Prep Kit contains reagents sufficient for 50 minipreps. All reagents can be stored at room temperature. Each kit includes the following components:

* Solution 1	10 ml	* Wash Solution (with ethanol)	80 ml
* Solution 2	10 ml	* Cyclo-Prep Spin Columns	50 each
* Solution 3	10 ml	* Collection Tubes	50 each

The protocol is easily accomplished using a pipettor and a bench-top microcentrifuge. Pelleted bacterial cells are simply resuspended in Solution 1, lysed with Solution 2, and the resulting lysate is neutralized with Solution 3. Following a brief centrifugation, the cleared lysate is loaded onto the Cyclo-Prep spin column, washed with Wash Solution containing ethanol, and eluted with H₂O or TE. The total time to prepare 12 plasmid DNA samples from overnight cultures is about 15 minutes.

For subsequent analysis of plasmid DNA, AMRESCO provides an extensive line of ancillary products and reagents. An agarose from our complete offering can be used for the visualization of your plasmid samples. For manual and automated sequencing protocols, AMRESCO's acrylamide products yield superior results. Order any of our high quality buffers and reagents to compliment your analytical application. Please contact an AMRESCO sales representative to receive additional details and pricing information.

1. To increase the yield of DNA by as much as 10-15%, a second elution step can be performed.
2. For automated fluorescent sequencing, an ethanol precipitation step is recommended following elution.

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PRODUCT INFORMATION

DIRECTIONS FOR USE

Cyclo-Prep™

Miniprep Plasmid DNA Purification Kit

Product Description:

Cyclo-Prep is a spin column-based kit for rapid purification of plasmid DNA from 1-2 ml liquid culture. In just 15 minutes, prepare ultra-pure plasmid DNA ($A_{260}/A_{280} > 1.8$) with a yield of 2-15 µg per ml of *E. coli* culture using your tabletop microcentrifuge. The DNA isolated from Cyclo-Prep is suitable for automated fluorescent sequencing, PCR, *in vitro* transcription, transformation and restriction enzyme digestion. The simple protocol provides an environmentally safe, convenient, and fast alternative to common phenol-based DNA purification procedures.

Kit Components:

The Cyclo-Prep Kit contains reagents sufficient for 50 minipreps. All reagents can be stored at room temperature. Each kit includes the following components:

* Solution 1	10ml	* Wash Solution (with ethanol)	80ml
* Solution 2	10ml	* Cyclo-Prep Spin Columns	50 each
* Solution 3	10ml	* Collection Tubes	50 each

Required Equipment: A micropipettor, bench-top microcentrifuge, and 1.5-1.7 ml microtuge tubes.

Protocol for Plasmid DNA Purification:

1. Transfer 1-2 ml of an overnight culture to a microcentrifuge tube. Pellet the bacterial cells by spinning at top speed (12-14,000 x g) for 30 sec. to 1 min. Remove the supernatant.
2. Add 200 µl of **Solution 1** to the pellet of cells. Pipette up and down (or vortex) to achieve complete resuspension.
3. Add 200 µl of **Solution 2**. Mix by gentle inversion of the tube 5-6 times.
4. Add 200 µl of **Solution 3**. Mix by gentle inversion of the tube 5-6 times.
5. Pellet the precipitate by centrifugation (12-14,000 x g) for 5 min. A white pellet will form at the bottom or along the side of the microcentrifuge tube.
6. Place a Cyclo-Prep **Spin Column** into a **Collection Tube**. Carefully remove the clear lysate from Step 6 and add it directly to the spin column.
7. Spin (12-14,000 x g) for 30 sec. Remove the spin column from the collection tube and discard the filtrate. The same collection tube can be used for the following step.
8. Add 700 µl of the **Wash Solution** to the spin column and spin (12-14,000 x g) for 30 sec. Remove the spin column from the collection tube and discard the filtrate. Spin for an additional 3 min. (12-14,000 x g) to remove residual traces of ethanol.
9. Remove the spin column and place it in a new microcentrifuge tube (not provided). Add 50-100 µl of preheated (60-70°C) H₂O or TE Buffer.
10. Elute the plasmid DNA by centrifugation (12-14,000 x g) for 30 sec.^{1,2} Store the eluted DNA at -20°C.

1. To increase the yield of DNA by as much as 10-15%, Steps 9-10 can be repeated.

2. For automated fluorescent sequencing, an ethanol precipitation step is recommended following elution.

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