



RetroPrep Ultrapure Miniprep Kit

6645 Nancy Ridge Dr.
San Diego, CA 92121
Tel: 800-retrogen (738-7643) Fax: 858-455-7987
Sales@retrogen.com www.retrogen.com



Retrogen, Inc. RetroPrep
Ultrapure Miniprep Kit

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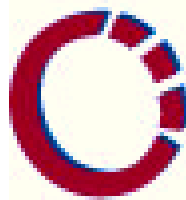
I. Description

Small-scale purifications of plasmid DNA, better known as minipreps, are commonly used in molecular biology procedures.

The Retrogen Miniprep DNA Purification System eliminates many of the problems associated with standard miniprep procedures, by providing a simple and reliable method for rapid isolation of plasmid DNA. This system can be used to isolate any plasmid, but works most efficiently when the plasmid is <20,000bp. The entire miniprep procedure can be completed in 30 minutes or less, depending on the number of samples processed. The purified plasmid can be used directly for automated fluorescent DNA sequencing or restriction enzyme digestion without further manipulation (when high copy number plasmids are used).

DNA is purified from the bacterial lysate using microcentrifugation or a vacuum manifold to force the cleared bacterial lysate through the Miniprep Spin Column and to wash the plasmid DNA. Figure 1 describes plasmid DNA isolation and purification using the Retrogen Miniprep DNA Purification System.

Storage and Stability: All Retrogen Miniprep components should be stored at 22°-25°C.



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II. Selection and Preparation of Plasmids and E. coli Strains

Plasmid DNA can be purified from 1-10ml overnight cultures of E. coli with the Retrogen Miniprep DNA Purification System. The yield of plasmid will vary depending on a number of factors, including the volume of bacterial culture, plasmid copy number, type of culture medium and the bacterial strain. The protocol presented here is for the isolation of plasmid DNA from E. coli.

A. Preparation of E. coli

Choose a single, well-isolated colony from a fresh Luria-Bertani (LB) agar plate (containing antibiotics) and use the colony to inoculate 1-10ml of LB medium (also containing antibiotics). The inoculated LB medium should be incubated overnight (12-16 hours) at 37°C. Do not incubate longer than 16 hours, as cell death and lysis may occur, resulting in plasmid loss. An OD600 reading of 2-4 ensures that cells have reached the proper growth density for harvesting and plasmid DNA isolation.

Antibiotics must be included in all culture media, both agar and liquid, to ensure propagation of only E. coli cells containing the plasmid of interest. The plasmid imparts antibiotic resistance to E. coli, allowing selection of plasmid-carrying bacteria over those that do not contain the plasmid. E. coli progeny that do not receive the plasmid during replication will grow, in medium without antibiotics, more quickly than those that contain the plasmid, and thus will overgrow the culture in the absence of the selective pressure of antibiotics. Use an antibiotic for which your plasmid contains a resistance gene.

III. Materials to Be Supplied by the User

(Solution compositions are provided in Section IV.)

LB agar plates containing antibiotic

LB medium containing antibiotic

ethanol (95%)

microcentrifuge capable of 14,000 x g

sterile 1.5ml microcentrifuge tubes

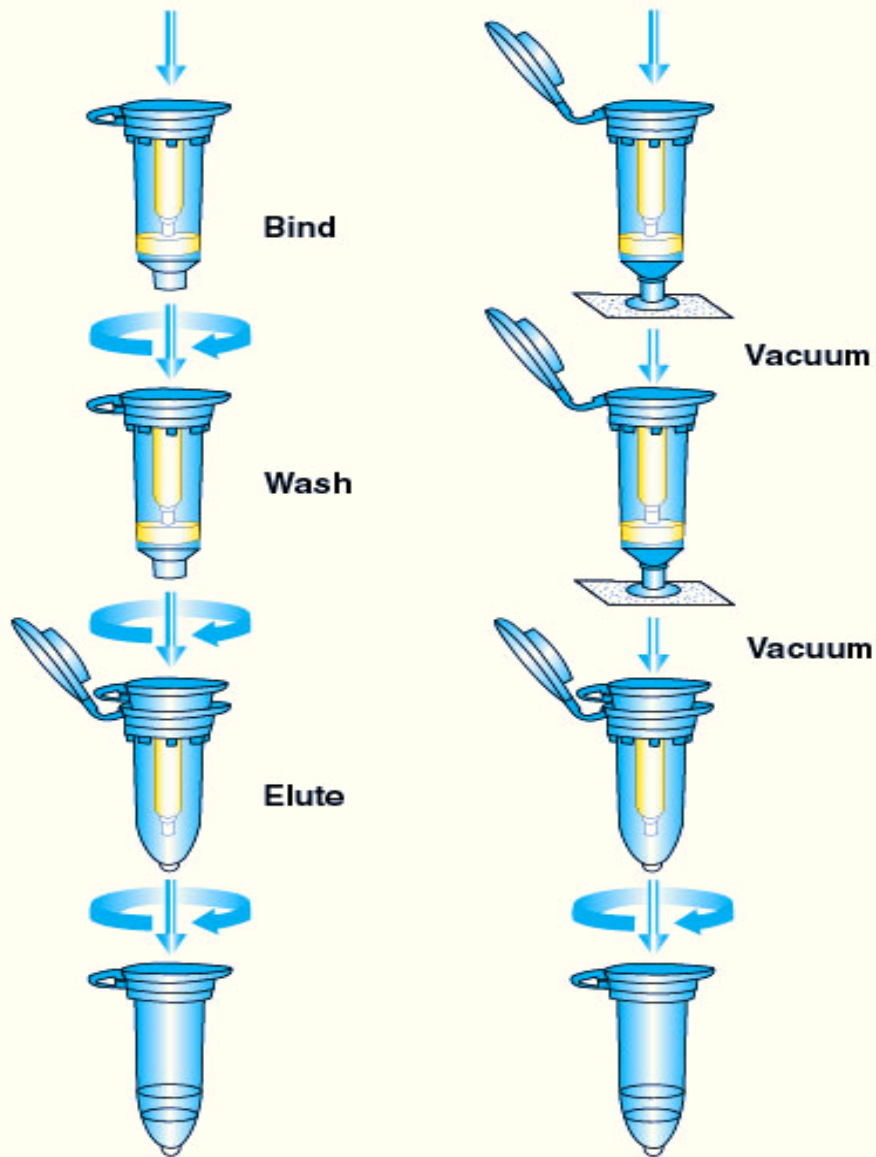
Tabletop centrifuge capable of 10,000 x g (for harvesting E. coli from 2-10ml of culture medium)



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**CLEARED
Bacterial Lysate**



Purified Plasmid DNA



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IV. Protocol

1. Harvest 1-5 ml of bacterial culture by centrifugation for 5 minutes at 10,000 x g. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.
2. Add 250 μ l of Cell Resuspension Buffer and completely resuspend the cell pellet by vortexing or pipetting. It is essential to thoroughly resuspend the cells.
3. Add 250 μ l of Cell Lysis Solution and mix by swirling the tube (do not vortex). Incubate until the cell suspension becomes slightly clear. Do not allow the lysis reaction to proceed for more than 5 minutes.
4. Add 350 μ l of Neutralization Solution and mix gently but thoroughly. Transfer the solution to a sterile 1.5ml microcentrifuge tube.
5. Centrifuge at 14,000 x g for 10 minutes. A white pellet will form.

A. By Centrifugation

During centrifugation, place 1 spin column into a 2 ml collection tube for each sample.

6. Apply the cleared supernatant from step 5 to the column either by decanting or pipetting. Avoid disturbing or transferring any of the white precipitate with the supernatant.
7. Centrifuge at 14,000 x g for 1 minute at room temperature. Remove the spin column from the collection tube and discard the flowthrough. Reinsert the spin column into the collection tube.
8. Add 650 μ l of Column Wash Solution (to which 95% Ethanol has been added as described in section IV).
9. Centrifuge at 14,000 x g for 1 minute at room temperature. Remove the spin column from the collection tube and discard the flowthrough. Reinsert the spin column into the collection tube.
10. Repeat the wash procedure by adding 250 μ l of Column Wash Solution.
11. Centrifuge at 14,000 x g for a full 2 minutes at room temperature. Remove the spin column from the collection tube and discard the flowthrough. This step is necessary to remove all traces of ethanol from the column membrane.



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12. Transfer the spin column to a sterile 1.5ml microcentrifuge tube being careful not to transfer any of the Column Wash Solution with the spin column. To elute DNA, add 25-50 μ l of nuclease free water to the center of the membrane. Let stand for 1 minute. Centrifuge for 1 minute at 14,000 x g.
13. Cap the microcentrifuge tube and store the purified plasmid DNA sample at -20°C or below.

B. By Vacuum

Attach one Miniprep Vacuum Adapter with Luer-Lok® fitting to one port of the manifold (refer to manufacturer's instructions). Insert a Retrogen Miniprep Spin Column into the Miniprep Vacuum Adapter until snugly in place.

6. Transfer the cleared bacterial lysate from Section IV, Step 5 into the Retrogen Miniprep Spin Column by decanting the liquid. If any of the precipitate is transferred to the Spin Column, pour the liquid and precipitate back into microcentrifuge tube and centrifuge again for 1 minute at 14,000 x g, then decant the liquid only into the Spin Column. The Retrogen Miniprep Spin Column can be reused, but only for the same preparation.
7. Apply a vacuum to pull the liquid through the Retrogen Miniprep Spin Column. When all liquid has been pulled through the column, release the vacuum.
8. Add 750 μ l of the Retrogen Miniprep Column Wash Solution, previously diluted with 95% ethanol (see Section V), to the Retrogen Miniprep Spin Column.
9. Apply a vacuum to pull the Retrogen Miniprep Column Wash Solution through the Retrogen Miniprep Spin Column. When all the liquid has been pulled through the column, release the vacuum.
10. Repeat the wash procedure, using 250 μ l of Column Wash Solution. Apply a vacuum to pull the liquid through the Retrogen Miniprep Spin Column.
11. When all the Column Wash Solution has been removed by vacuum, turn off the vacuum and transfer the Retrogen Miniprep Spin Column to a 2ml Collection Tube. Centrifuge at 14,000 x g for 2 minutes to remove any residual Column Wash Solution. Discard the 2ml Collection Tube and any liquid collected during this step.
12. Transfer the Retrogen Miniprep Spin Column to a new, sterile 1.5ml microcentrifuge tube.



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V. Buffers and Solutions

Cell Resuspension Solution

Tris-HCl, pH 7.5
EDTA
RNase A

Cell Lysis Solution

NaOH
SDS

Neutralization Solution

Guanidine hydrochloride
Potassium acetate
Glacial acetic acid

Final pH is approximately 4.2.

Column Wash Solution (100 mL buffer concentrate)

Potassium acetate
Tris-HCl, pH 7.5

Add 170ml of 95% ethanol for a total volume of 270 mL.