StrataPrep Plasmid Miniprep Kit

Instruction Manual

Catalog #400761, #400763

Revision B1

For Research Use Only. Not for use in diagnostic procedures.

400761-12

Agilent Technologies

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

Email

techservices@agilent.com

World Wide Web

www.genomics.agilent.com

Telephone

Location	Telephone
United States and Canada	800 227 9770
Austria	01 25125 6800
Benelux	02 404 92 22
Denmark	45 70 13 00 30
Finland	010 802 220
France	0810 446 446
Germany	0800 603 1000
Italy	800 012575
Netherlands	020 547 2600
Spain	901 11 68 90
Sweden	08 506 4 8960
Switzerland	0848 8035 60
UK/Ireland	0845 712 5292
All Other Countries	Please visit *www.agilent.com/genomics/contactus*

StrataPrep Plasmid Miniprep Kit

Contents

StrataPrep Plasmid Miniprep Kit

MATERIALS PROVIDED

Materials provided	Quantity	
	Catalog #400761[a]	Catalog #400763[b]
Solution 1	6 ml	30 ml
Solution 2	6 ml	30 ml
Solution 3	8 ml	40 ml
Wash Buffer (2×)	25 ml	125 ml
Nuclease Removal Buffer (1×)	40 ml	200 ml
Microspin cups[c]	50	250
Receptacle tubes (2 ml)	50	250

[a] Contains sufficient reagents for fifty 50-µl minipreps.

[b] Contains sufficient reagents for two hundred fifty 50-µl minipreps.

[c] The capacity of the microspin cup is ~0.8 ml.

Caution *The chaotropic salt in Solution 3 is an irritant.*

STORAGE CONDITIONS

All Components: Room temperature

ADDITIONAL MATERIALS REQUIRED

Elution buffer (see *Preparation of Reagents*)
Ethanol (100%)
Microcentrifuge
Microcentrifuge tubes (1.5-ml)

Revision B1

INTRODUCTION

The StrataPrep plasmid miniprep kit provides a rapid, phenol-free method for purifying plasmid DNA from bacterial cultures. The method employs a modification of the alkaline method of cell lysis[1] and a microspin cup with a silica-based fiber matrix that binds DNA in the presence of a chaotropic salt.[2] The sample is combined with a ribonuclease-containing solution, a cell lysis solution, and a DNA-binding solution and is then transferred to a microspin cup that is seated inside a receptacle tube. The plasmid DNA binds to the fiber matrix in the microspin cup. The contaminants are then washed from the microspin cup with a wash buffer. The purified plasmid DNA is eluted from the fiber matrix with a low-ionic-strength buffer and captured in a microcentrifuge tube. The result is purified plasmid DNA that is ready for restriction digestion, ligation, and sequencing reactions.

PURIFYING PLASMID DNA

1. Inoculate a culture medium with a single bacterial colony and add the appropriate antibiotic.

2. Incubate the cell culture 16–24 hours at 37°C with vigorous shaking.

3. Aliquot 1.5 ml of the cell culture into a 1.5-ml microcentrifuge tube.

4. Spin the tube in a microcentrifuge at maximum speed for 1 minute. After centrifugation, remove and discard the supernatant.

5. Add 100 µl of solution 1 to the microcentrifuge tube. Vortex the tube or pipet the cell–solution 1 mixture up and down to resuspend and completely disperse the cells.

6. Add 100 µl of solution 2 to the microcentrifuge tube. Mix the contents by inverting the tube several times (**do not vortex the tube**).

   Note *Keep solution 2 capped tightly before and after use.*

7. Add 125 µl of solution 3 to the microcentrifuge tube. Mix the contents by inverting the tube several times (**do not vortex the tube**).

   Caution *Exercise caution when using solution 3. The chaotropic salt in solution 3 is an irritant.*

8. Spin the tube in a microcentrifuge at maximum speed for 5 minutes. A compact white pellet forms. The supernatant contains the plasmid DNA.

9. Taking care to remove as little of the precipitated material as possible, decant the supernatant or transfer the supernatant with a pipet to a microspin cup that is seated in a 2-ml receptacle tube. Snap the cap of the 2-ml receptacle tube onto the top of the microspin cup. Discard the pellet and the microcentrifuge tube.

10. Spin the sample in a microcentrifuge at maximum speed for 30 seconds.

    Note *The plasmid DNA is retained in the fiber matrix of the microspin cup. The binding capacity of the microspin cup is ~20 µg of plasmid DNA.*

11. Open the cap of the 2-ml receptacle tube, remove the microspin cup from the receptacle tube, and discard the liquid. Replace the microspin cup in the receptacle tube.

Important *If the* E. coli *host strain is* endA1$^-$ *(i.e., lacks the gene for DNA-specific endonuclease I), proceed with step 13. If the host strain is* endA1$^+$ *(i.e., contains the gene for DNA-specific endonuclease I), perform step 12 and then continue with the remainder of the protocol.*

12. Wash the endonucleases from the sample according to the following protocol:

    a. Add 750 µl of Nuclease Removal Buffer to the microspin cup. Snap the cap of the 2-ml receptacle tube onto the top of the microspin cup.

    b. Spin the sample in a microcentrifuge at maximum speed for 30 seconds.

    c. Remove the microspin cup and discard the filtrate in the microcentrifuge tube. Place the microspin cup back in the 2-ml receptacle tube and snap the cap of the receptacle tube onto the top of the microspin cup.

    d. Spin the sample in a microcentrifuge at maximum speed for an additional 30 seconds.

    e. Remove the microspin cup and discard the filtrate.

    f. Replace the microspin cup in the receptacle tube.

13. Prepare the 1× wash buffer by adding an equal volume of 100% (v/v) ethanol to the container of the 2× wash buffer (25 ml of 100% ethanol for catalog #400761 and 125 ml of 100% ethanol for catalog #400763). After adding the ethanol, mark the box on the label on the container—*[✓] 1× (Ethanol Added)*. Store the 1× wash buffer at room temperature.

14. Add 750 µl of 1× wash buffer to the microspin cup. Snap the cap of the 2-ml receptacle tube onto the top of the microspin cup.

15. Spin the sample in a microcentrifuge at maximum speed for 30 seconds.

16. Remove the microspin cup and discard the filtrate. Replace the microspin cup in the 2-ml receptacle tube and snap the cap of the receptacle tube onto the top of the microspin cup.

17. Spin the sample in a microcentrifuge at maximum speed for an additional 30 seconds. On removal from the centrifuge, make sure that all of the wash buffer is removed from the microspin cup.

18. Transfer the microspin cup to a fresh 1.5-ml microcentrifuge tube[‖] and discard the 2-ml receptacle tube.

19. Load 50 µl of elution buffer (see *Preparation of Reagents*) directly on top of the fiber matrix at the bottom of the microspin cup.

    Note *For eluting DNA from the microspin cup, use a low-ionic-strength buffer (≤10 mM in concentration, pH 7–9) or sterile deionized water. For most applications 10 mM Tris base (pH adjusted to 8.5 with HCl) is recommended; however, TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA) may be used for applications in which EDTA will not interfere with subsequent reactions.*

20. Incubate the sample at room temperature for 5 minutes.

    Note *Maximum recovery of the plasmid DNA from the microspin cup depends on the volume of the elution buffer added to the microspin cup, the placement of the elution buffer into the microspin cup, and the incubation time. Maximum recovery is obtained if not less than 50 µl of elution buffer is added directly onto the fiber matrix at the bottom of the microspin cup and the sample is incubated for 5 minutes.*

21. Snap the cap of the 1.5-ml microcentrifuge tube onto the top of the microspin cup and spin the sample in a microcentrifuge at maximum speed for 30 seconds.

22. Open the lid of the microcentrifuge tube and discard the microspin cup.

    Note *The purified plasmid DNA is in the bottom of the 1.5-ml microcentrifuge tube. Snap the lid of the microcentrifuge tube closed to store the purified plasmid DNA.*

    The binding capacity of the microspin cup is ~20 µg of plasmid DNA.

[‖] 1.5-ml flat snap cap microcentrifuge tubes from Continental Laboratory Products, Inc. are recommended.

TROUBLESHOOTING

Observation	Suggestion
The plasmid DNA is contaminated with chromosomal DNA	Chromosomal DNA is sheared by vortexing the tube to mix the contents after adding solution 2 or solution 3. Mix the contents of the tube by gentle inversion; do not vortex
The plasmid DNA is not supercoiled	Plasmid DNA is nicked by nuclease contamination. Include the wash procedure in step 12 in the protocol
The cells are not lysed with the addition of solution 2	Solution 2 is degraded by reaction with air over time. Keep the container of solution 2 capped tightly before and after use; make a fresh solution if necessary
The DNA floats out of the wells of the agarose gel	Make sure that all the wash buffer is removed from the microspin cup before adding the elution buffer to avoid ethanol contamination

PREPARATION OF REAGENTS

Elution Buffer

10 mM Tris base
Adjust pH to 8.5 with HCl
or
10 mM Tris base
1 mM EDTA
Adjust pH to 8.0 with HCl
or
Sterile ddH_2O

Solution 1

50 mM Tris HCl (pH 7.5)
10 mM EDTA
50 µg/ml of RNase A

Solution 2

0.2 M NaOH
1% (w/v) SDS

Note *Store tightly capped before and after use.*

2× Wash Buffer

10 mM Tris HCl (pH 7.5)
100 mM NaCl
2.5 mM EDTA

REFERENCES

1. Birnboim, H. C. and Doly, J. (1979) *Nucleic Acids Res* 7(6):1513-23.
2. Vogelstein, B. and Gillespie, D. (1979) *Proc Natl Acad Sci U S A* 76(2):615-9.

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.chem.agilent.com/en-US/search/library/Pages/MSDSSearch.aspx*. MSDS documents are not included with product shipments.

StrataPrep Plasmid Miniprep Kit

QUICK-REFERENCE PROTOCOL

- Inoculate a culture medium with a bacterial colony, add the appropriate antibiotic, and incubate the cell culture overnight at 37°C with vigorous shaking
- Aliquot 1.5 ml of the cell culture into a 1.5-ml microcentrifuge tube
- Spin the sample in a microcentrifuge for 1 minute and discard the supernatant
- Add 100 µl of solution 1 to the tube and vortex or pipet the cell–solution 1 mixture to resuspend and disperse the cells
- Add 100 µl of solution 2 to the tube and mix by inversion (**Do not vortex**)
- Add 125 µl of solution 3 to the tube and mix by inversion (**Do not vortex**)
- Spin the sample in a microcentrifuge for 5 minutes
- Decant or pipet the supernatant, which contains the plasmid DNA, into a microspin cup that is seated in a 2-ml receptacle tube
- Spin the sample in a microcentrifuge for 30 seconds
- Remove the microspin cup, discard the filtrate, and replace the microspin cup in the receptacle tube
- Perform the optional wash step
    - Add 750 µl of 1× Nuclease Removal Buffer to the microspin cup
    - Spin the sample in a microcentrifuge for 30 seconds and discard the filtrate
    - Spin the sample in a microcentrifuge again and discard the filtrate
- Add 750 µl of 1× wash buffer to the microspin cup and spin the sample in a microcentrifuge for 30 seconds, discard the filtrate, spin the sample again, and discard the filtrate
- Transfer the microspin cup to a fresh 1.5-ml microcentrifuge tube
- Add 50 µl of elution buffer directly onto the top of the fiber matrix of the microspin cup and incubate the sample for 5 minutes
- Spin the sample in a microcentrifuge for 30 seconds and discard the microspin cup

Note *The purified plasmid DNA is in the bottom of the 1.5-ml microcentrifuge tube.*