StrataPrep DNA Gel Extraction Kit

INSTRUCTION MANUAL

Catalog #400766 and #400768

Revision B.0

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400766-12

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StrataPrep DNA Gel Extraction Kit

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MATERIALS PROVIDED

Materials provided	Quantity	
	Catalog #400766[a]	**Catalog #400768[b]**
DNA extraction buffer	20 ml	100 ml
Wash buffer (2×)	25 ml	125 ml
Microspin cups[c]	50	250
Receptacle tubes (2 ml)	50	250

[a] Contains enough reagents for 50 gel extractions.

[b] Contains enough reagents for 250 gel extractions.

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

Caution *The chaotropic salt in the DNA extraction buffer is an irritant.*

STORAGE CONDITIONS

All Components: Room temperature

ADDITIONAL MATERIALS REQUIRED

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

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INTRODUCTION

The StrataPrep DNA gel extraction kit is a rapid method for extracting gel-fractionated DNA from agarose gels. The method employs a microspin cup that contains a silica-based fiber matrix. In the presence of a chaotropic salt, the agarose is dissolved and the DNA binds to the fiber matrix.[1] Following gel fractionation of the DNA, the desired fragment is cut from the gel, placed in a microcentrifuge tube, combined with the DNA extraction buffer, incubated at 50°C, and transferred to a microspin cup that is seated inside a receptacle tube. The 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 DNA is eluted from the fiber matrix with a low-ionic-strength buffer and captured in a microcentrifuge tube. Double-stranded DNA ≥100 bp is retained. This simple method of DNA extraction eliminates the need for manipulation of resins, toxic phenol–chloroform extractions, and time-consuming ethanol precipitations. The result is purified DNA that is ready for restriction digestion, ligation, and probe labeling.

GEL EXTRACTION PROTOCOL

Note *The following protocol is for the recovery of DNA from a conventional 1% agarose gel (TAE or TBE). If the gel concentration is ≥2%, use twice the volume of DNA extraction buffer for the volume of gel described in the following procedure.*

1. Add 300 µl of DNA extraction buffer for each 100 µl of gel volume [a gel slice with dimensions of 0.8 cm × 0.3 cm × 0.5cm = 0.12 cm^3, ~120 µl (by volume) or ~120 mg (by weight)] to a 1.5-ml microcentrifuge tube.

2. Heat the mixture at 50°C for at least 10 minutes with occasional mixing. Be sure that the gel is completely dissolved before continuing to the next step.

3. Transfer the mixture to a microspin cup that is seated in a 2-ml receptacle tube (exercise caution to avoid damaging the fiber matrix). Snap the cap of the 2-ml receptacle tube onto the top of the microspin cup.

4. Spin the tube in a microcentrifuge at maximum speed for 30 seconds.

   Note *The DNA is retained in the fiber matrix of the microspin cup. The binding capacity of the microspin cup is ~10 µg.*

5. Prepare the 1× wash buffer by adding an equal volume of 100% ethanol to the container of 2× wash buffer: 25 ml of 100% ethanol for catalog #400766 **or** 125 ml of 100% ethanol for catalog #400768. After adding the ethanol, mark the label on the container as suggested: *[✓] 1× (Ethanol Added)*. Store the 1× wash buffer at room temperature.

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

7. Add 750 µl of 1× wash buffer to the microspin cup. Snap the cap of the receptacle tube onto the top of the microspin cup.

8. Spin the tube in a microcentrifuge at maximum speed for 30 seconds.

9. Open the cap of the 2-ml receptacle tube, remove and retain the microspin cup, and discard the wash buffer.

10. Place the microspin cup back in the 2-ml receptacle tube and snap the cap of the receptacle tube onto the microspin cup.

11. Spin the tube in a microcentrifuge at maximum speed for 30 seconds. On removal from the microcentrifuge, make sure that all of the wash buffer is removed from the microspin.

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

13. Add 50 µl of elution buffer directly onto the 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 (10 mM Tris HCl, pH 8.0, 1mM EDTA) may be used for applications in which EDTA will not interfere with subsequent reactions.*

14. Incubate the tube at room temperature for 5 minutes.

    Note *Maximum recovery of the DNA from the microspin cup depends on the pH, ionic strength, and 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 tube is incubated for 5 minutes.*

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

16. Open the lid of the 1.5-ml microcentrifuge tube and discard the microspin cup.

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

    An 80% recovery is expected from DNA that is 250bp–9kb; a 50% recovery is expected for longer DNA up to 23kb.

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

TROUBLESHOOTING

Observation	Suggestion
Low recovery of the desired DNA	Gel may not be completely dissolved following addition of the DNA extraction buffer. Verify that the volume of the DNA extraction buffer is correct for the volume of the agarose gel. If the gel concentration is ≥2%, add twice the volume of DNA extraction buffer
	Ensure that the 2× wash buffer is diluted with an equal volume of 100% ethanol so that the DNA is washed and retained on the microspin cup. Prepare 1× wash buffer by adding an equal volume of 100% ethanol to the 2× wash buffer
	Do not use a solution of high ionic strength or low pH as the elution buffer. Instead, use a low-ionic-strength (≤10 mM) buffer, pH 7–9
	Do not dispense the elution buffer down the side of the microspin cup. Add the elution buffer directly onto the fiber matrix of the microspin cup to ensure complete coverage of the membrane
	Incubate the tube for 5 minutes after adding the elution buffer
The DNA floats out of the well of the agarose gel	Make sure that the 1× wash buffer is completely 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

2× Wash Buffer

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

REFERENCE

1. Vogelstein, B. and Gillespie, D. (1979) *Proc Natl Acad Sci U S A* 76(2):615-9.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

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QUICK-REFERENCE PROTOCOL

- Add the appropriate volume of DNA extraction buffer to the gel slice
- Heat at 50°C for at least 10 minutes (until gel is completely dissolved)
- Transfer the DNA-DNA extraction buffer mixture into a microspin cup that is seated in a 2-ml receptacle tube
- Spin the tube in a microcentrifuge for 30 seconds. Open the cap of the 2-ml receptacle tube, remove and retain the microspin cup, and discard the liquid
- Prepare the 1× wash buffer by adding an equal volume of 100% ethanol to the container of 2× wash buffer
- Add 750 µl of 1× wash buffer to the microspin cup
- Spin the tube in the microcentrifuge for 30 seconds
- Open the cap of the 2-ml receptacle tube, remove and retain the microspin cup, and discard the wash buffer
- Place the microspin cup back in the 2-ml receptacle tube and snap the cap of the 2-ml receptacle tube onto the microspin cup
- Spin the tube in a microcentrifuge for 30 seconds
- Transfer the microspin cup to a fresh 1.5-ml microcentrifuge tube and discard the 2-ml receptacle tube
- Add 50 µl of elution buffer directly onto the fiber matrix at the bottom of the microspin cup
- Incubate the tube at room temperature for 5 minutes
- To collect the DNA, spin the tube in a microcentrifuge for 30 seconds
- Open the lid of the 1.5-ml microcentrifuge tube and discard the microspin cup

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