StrataPrep PCR Purification Kit

INSTRUCTION MANUAL

Catalog #400771 and #400773

Revision A

For In Vitro Use Only

400771-12

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StrataPrep PCR Purification Kit

CONTENTS

Materials Provided 1
Storage Conditions 1
Additional Materials Required 1
Introduction 2
Purifying PCR Products 3
Troubleshooting 5
Preparation of Media and Reagents 6
Appendix: Purifying Dye-Coupled cDNA 6
Reference 9
Endnotes 9
Quick-Reference Protocol for Purifying PCR Products 11
Quick-Reference Protocol for Purifying Dye-Coupled cDNA 12

StrataPrep PCR Purification Kit

MATERIALS PROVIDED

Materials provided	Quantity	
	Catalog #400771[a]	Catalog #400773[b]
DNA-binding solution	5 ml	25 ml
Wash buffer (5×)	10 ml	50 ml
Microspin cups[c]	50	250
Receptacle tubes (2 ml)	50	250

[a] Contains sufficient reagents to purify fifty 50-µl PCR reactions or twenty-five 100-µl dye-coupled cDNA reactions.

[b] Contains sufficient reagents to purify two hundred fifty 50-µl PCR reactions or one hundred twenty-five 100-µl dye-coupled cDNA reactions.

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

STORAGE CONDITIONS

All Components: Room temperature

Caution *The chaotropic salt in the DNA-binding solution is an irritant.*

ADDITIONAL MATERIALS REQUIRED

Elution buffer (see *Preparation of Media and Reagents*)
Ethanol (100%, 70%, and 75%)
Microcentrifuge
Microcentrifuge tubes (1.5 ml)

Revision A

INTRODUCTION

The StrataPrep PCR purification kit provides a rapid method to separate PCR products from PCR primers, unincorporated nucleotides, buffer components, and enzymes. The method employs a microspin cup that contains a silica-based fiber matrix. In the presence of a chaotropic salt, DNA binds to the fiber matrix.[1]

Following PCR amplification, the PCR product is combined with a DNA-binding solution and transferred to a microspin cup that is seated inside a receptacle tube. The PCR product binds to the fiber matrix in the microspin cup. The contaminants are then washed from the microspin cup with a wash buffer. The purified PCR products are 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 purification eliminates tedious manipulation of resins, the toxic phenol–chloroform extraction, and the time-consuming ethanol precipitation used in other DNA purification methods. The result is a highly purified PCR product that is ready for restriction digestion, ligation, and sequencing reactions.

In addition to the purification of PCR products, the StrataPrep PCR purification kit provides a rapid method to separate fluorescence-labeled cDNA from uncoupled or unincorporated fluorescent dye, primers, buffer components, and enzymes. The method can be used to purify fluorescence-labeled cDNA from either direct or indirect fluorescent cDNA labeling protocols. The purified cDNA is ready for use in microarray hybridizations.

Note *There are two separate protocols included in this manual, optimized for different purification requirements. For purifying PCR products, see* Purifying PCR Products*, below. For separating fluorescence-labeled cDNA from uncoupled or unincorporated fluorescent dye, see* Appendix.

PURIFYING PCR PRODUCTS

Use this protocol for purifying **PCR products** only. If purifying fluorescence-labeled cDNA from uncoupled or unincorporated fluorescent dye, see *Appendix*.

1. Add a volume of DNA-binding solution equal to the volume of the aqueous portion of the PCR product to the microcentrifuge tube containing the PCR product and mix the two components.

   Note *Mineral oil from the PCR reaction does not affect the purification process (Avoiding the mineral oil overlay, however, is recommended.). Do not include the volume of the mineral oil overlay when calculating the quantity of DNA-binding solution to add to the PCR product.*

2. Using a pipet, transfer the PCR product–DNA-binding-solution mixture to a microspin cup that is seated in a 2-ml receptacle tube. (Exercise caution to avoid damaging the fiber matrix with the pipet tip.) Snap the cap of the 2-ml receptacle tube onto the top of the microspin cup.

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

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

4. Open the cap of the 2-ml receptacle tube, remove and retain the microspin cup, and discard the DNA-binding solution.

5. Prepare the 1× wash buffer by adding the following to the container of the 5× wash buffer: 40 ml of 100% (v/v) ethanol for catalog #400771 or 200 ml of 100% (v/v) ethanol for catalog #400773. After adding the ethanol, mark the box on the label on the container—*[ ] 1× (Ethanol Added)*. Store the 1× wash buffer at room temperature.

6. Open the cap of the 2-ml receptacle tube and 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.

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

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

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

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

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

12. Add 50 µl of elution buffer directly onto the top of the fiber matrix at the bottom of the microspin cup. Avoid touching the fiber matrix with the pipet tip.

    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 (see* Preparation of Media and Reagents*).*

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

    Note *Maximum recovery of the PCR product from the microspin cup depends on the pH, the ionic strength, and 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 the elution buffer is ≤10 mM in concentration with pH 7–9, 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.*

14. 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.

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

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

    The binding capacity of the microspin cup is ~10 µg of DNA.

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

TROUBLESHOOTING

Observation	Suggestion
Low recovery of the PCR product	Verify that the PCR product is synthesized by running a portion of the unpurified PCR product on an agarose gel
	Ensure that wash buffer at the correct concentration is used during the washes. Concentrated wash buffer will elute the PCR product from the microspin cup. To prepare the 1× wash buffer, add four volumes of 100% (v/v) ethanol to the 5× wash buffer (see step 5 of the PCR product purification protocol)
	Elute the PCR product using a low-ionic-strength (≤10 mM) buffer, pH 7–9
	Ensure that the PCR product is eluted by the addition of not less than 50 µl of elution buffer to the microspin cup
	Ensure that the elution buffer is added directly onto the fiber matrix of the microspin cup and completely covers the membrane
	Incubate the tube for 5 minutes after adding the elution buffer
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. The wash buffer contains ethanol which can cause the DNA to float out of the wells of an agarose gel
Low recovery of the fluorescence-labeled cDNA	Ensure that wash buffer at the correct concentration is used during the washes. Concentrated wash buffer will elute the labeled cDNA from the microspin cup. Prepare the wash buffer by adding three volumes of 100% (v/v) ethanol to 1 volume of water.
	Ensure that the final DMSO concentration of the sample to be purified is 5% or less
	Ensure that the labeled cDNA is eluted by the addition of not less than 50 µl of elution buffer to the microspin cup
	Ensure that the elution buffer is added directly onto the fiber matrix of the microspin cup and completely covers the membrane
	Incubate the tube for 5 minutes after adding the elution buffer
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. The wash buffer contains ethanol which can cause the DNA to float out of the wells of an agarose gel

PREPARATION OF MEDIA AND REAGENTS

Elution Buffer	5× Wash Buffer (for PCR product purification)
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	10 mM Tris HCl (pH 7.5) 100 mM NaCl **1x Wash Buffer (for cDNA purification)** 75 ml 100% (v/v) ethanol 25 ml dH_2O, molecular biology grade, DNase- and RNase-free

APPENDIX: PURIFYING DYE-COUPLED cDNA

Use this protocol for purifying **dye-coupled cDNA** only.

1. Adjust the labeled cDNA volume to 100 µl with water. If DMSO is present in the reaction adjust the volume to a final DMSO concentration of 5% or less.

   Note *If the final volume is greater than 250 µl divide your reaction in half and use 2 PCR purification columns.*

2. Combine 100 µl of DNA-binding solution and 100 µl of 70% (v/v) EtOH. Mix well by vortexing, It is important that the two solutions are well mixed prior to adding it to the labeled cDNA.

3. Add the DNA-binding solution/EtOH mixture to the labeled cDNA and mix by vortexing.

   Note *If the final volume of labeled cDNA is greater than 100 µl, add 2 volumes of the DNA-binding solution/EtOH mixture (for every 100 µl reaction add 200 µl of the DNA-binding solution/EtOH mixture)*

4. Using a pipet, transfer the mixture to a microspin cup that is seated in a 2-ml receptacle tube. (Avoid damaging the fiber matrix with the pipet tip when pipetting the mixture) Snap the cap of the 2-ml receptacle tube onto the top of the microspin cup.

   Note *To ensure proper sample flow, use the receptacle tube that is provided in the StrataPrep PCR Purification Kit. Do not substitute another tube.*

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

   Note *The labeled cDNA is retained in the fiber matrix of the microspin cup.*

6. Open the cap of the 2-ml receptacle tube, remove and retain the microspin cup, and discard the DNA-binding solution containing the uncoupled dye.

7. Combine 100 µl of the DNA-binding solution and 100 µl of 70% (v/v) EtOH. Mix well by vortexing. It is important that the two solutions are well mixed prior to use.

8. Add the DNA-binding solution/EtOH mixture to the microspin cup. Snap the cap of the receptacle tube onto the top of the microspin cup.

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

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

11. Add 750 µl of 75% ethanol to the microspin cup. Snap the cap of the receptacle tube onto the top of the microspin cup.

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

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

14. Repeat steps 11–13.

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

16. Spin the tube in a microcentrifuge at maximum speed for 30 seconds. On removal from the centrifuge, inspect the tube to ensure that no residual wash buffer remains in the microspin cup.

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

18. Add 50 µl of 10 mM Tris base, pH 8.5 directly onto the top of the fiber matrix at the bottom of the microspin cup. Avoid touching the fiber matrix with the pipet tip.

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

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

    Note *Maximum recovery of the labeled cDNA from the microspin cup depends on the pH, the ionic strength, and 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 the elution buffer is ≤10 mM in concentration with pH 7–9, 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*

20. 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.

21. Open the lid of the microcentrifuge tube and recover the flow through containing the purified labeled cDNA.

22. Elute additional labeled cDNA by pipetting the flow through back onto the fiber matrix of the same microspin cup.

23. Re-seat the spin cup on the same 2-ml receptacle tube used in the first-pass elution.

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

25. 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.

26. Open the lid of the microcentrifuge tube and recover the flow through containing the purified labeled cDNA.

27. Harvest one final elution from the microspin cup by repeating steps 22–26.

28. Open the lid of the microcentrifuge tube and recover the final elution flow through containing the purified labeled cDNA.

It may be necessary to concentrate the purified cDNA prior to microarray hybridization experiments. Consult the hybridization protocol to be used for guidelines on the cDNA concentration required. If not proceeding directly to a microarray hybridization experiment, it is possible to store the purified, fluorescence-labeled cDNA (immediately following elution or following elution and concentration) at 4°C for up to 7 days.

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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StrataPrep PCR Purification Kit

Catalog #400771 and #400773

QUICK-REFERENCE PROTOCOL FOR PURIFYING PCR PRODUCTS

- Add an equal volume of DNA-binding solution to the PCR product and mix the two components
- Transfer the PCR product–DNA-binding-solution mixture to 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 receptacle tube, remove and retain the microspin cup, and discard the DNA-binding solution.
- Prepare the 1× wash buffer by adding the following to the container of the 5× wash buffer: 40 ml of 100% (v/v) ethanol for catalog #400771 or 200 ml of 100% (v/v) ethanol for catalog #400773
- Add 750 µl of 1× wash buffer to the microspin cup
- Spin the tube in a microcentrifuge for 30 seconds
- Open the cap of the 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 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 PCR product, spin the tube in a microcentrifuge for 30 seconds
- Open the lid of the microcentrifuge tube and discard the microspin cup

Note *The purified PCR product is in the bottom of the 1.5-ml microcentrifuge tube*

QUICK-REFERENCE PROTOCOL FOR PURIFYING DYE-COUPLED cDNA

- Add 200 µl of the DNA-binding solution/EtOH mixture to 100 µl of labeled cDNA and mix the two components
- Transfer the labeled cDNA/DNA-binding-solution mixture to 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 receptacle tube, remove and retain the microspin cup, and discard the DNA-binding solution.
- Add 200 µl of an equal mixture of DNA-binding solution and 70% EtOH to the microspin cup
- Spin the tube in a microcentrifuge for 30 seconds
- Open the cap of the receptacle tube, remove and retain the microspin cup, and discard the wash buffer
- Place the microspin cup back in the 2-ml receptacle tube
- Add 750 µl of 75% EtOH to the microspin cup
- Spin the tube in a microcentrifuge for 30 seconds
- Repeat the 75% EtOH wash step
- Discard the wash buffer after the final wash step and dry the fiber matrix by centrifugation 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 Tris base, pH 8.5 directly onto the fiber matrix at the bottom of the microspin cup
- Incubate the tube at room temperature for 5 minutes
- To collect the labeled cDNA, spin the tube in a microcentrifuge for 30 seconds
- To maximize elution of the labeled cDNA, pass the same eluate over the fiber matrix two additional times, using 30 second microcentrifugation steps and 5 minute incubations steps each time

Note *After the final spin, the purified cDNA is in the bottom of the 1.5-ml microcentrifuge tube*