StrataPrep 96 PCR Purification Kit

INSTRUCTION MANUAL

Catalog #400775 (2 Plates)
Revision C.0

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400775-12

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StrataPrep 96 PCR Purification Kit

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MATERIALS PROVIDED

Materials Provided	Quantity[a]
DNA-binding solution	25 ml
PCR wash buffer (5×)	50 ml
StrataPrep 96-well binding plates	2
96-well collection plates	4
Plate sealers	4
Storage Mats	2
Elution buffer	25 ml

[a] Contains sufficient reagents to purify one hundred ninety-two 50-µl PCR reactions.

STORAGE CONDITIONS

All Components: Room temperature

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

ADDITIONAL MATERIALS REQUIRED

Ethanol (100%)
Centrifuge adaptable for use with 96-deep well plate rotor
Vacuum manifold with waste tray
Multichannel pipet

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INTRODUCTION

The StrataPrep 96 PCR purification kit provides a rapid method to simultaneously purify 96 PCR products from PCR primers, unincorporated nucleotides, buffer components, and enzymes. This simple method of DNA purification eliminates the need for tedious manipulation of resins, toxic phenol–chloroform extraction, and the time-consuming ethanol precipitation used in other DNA purification methods.

Following PCR amplification, the PCR products are combined with a DNA-binding solution and transferred to a StrataPrep 96-well binding plate. Each well of the StrataPrep binding plate contains a silica-based fiber matrix. A chaotropic salt present in the DNA-binding solution enables the DNA to bind to the fiber matrix.[1] Unbound contaminants are then washed from each well. Purified PCR products are eluted from the fiber matrix using a low ionic strength buffer and captured in a 96-well collection plate. Double-stranded DNA ≥100 bp in length is retained in the collection plate. The result is a highly-purified PCR product that can be used in restriction digestion, ligation, and sequencing reactions.

PROTOCOL

1. Mix the aqueous portion of each PCR product with an equal volume of DNA-binding solution.

   Note *Mineral oil from the PCR process does not affect the purification process however, avoiding the mineral oil overlay 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. Place a StrataPrep 96-well binding plate on a vacuum manifold that contains a waste tray at the bottom. Using a multichannel pipet, transfer the PCR product/DNA-binding solution mixtures into the wells of the binding plate.

   Note *If some of the 96 wells do not contain samples, seal the tops of the empty wells with tape or cover the entire plate and carefully cut slits over the wells containing samples. Take care not to cross-contaminate the samples when cutting slits in the tape.*

3. Apply 400 mbar of vacuum to the binding plate until each well is dry. The vacuum force may decrease as the wells become dry (~1 minute). Continue the vacuum for an additional 5 minutes after the wells appear dry.

   Note *The PCR products are retained in the fiber matrix of the binding plate. The binding capacity of each well of the binding plate is ~5 µg of DNA (well volume is 750 µl).*

4. Prepare 1× PCR wash buffer by adding 4 volumes of 100% (v/v) ethanol to the 5× PCR wash buffer container. After adding the ethanol, check the box on the label: *[ ] 1× (Ethanol Added)*. Store the 1× PCR wash buffer tightly sealed at room temperature.

5. Add 750 µl of 1× PCR wash buffer to each well of the binding plate.

6. Apply 400 mbar of vacuum until each well is dry (~1 minute).

7. Remove the binding plate from the vacuum manifold and discard the wash solution from the waste tray. Replace the waste tray inside and the binding plate on top of the vacuum manifold and apply 400 mbar of vacuum to the binding plate for an additional 5 minutes.

8. Release the vacuum and remove the binding plate from the manifold. Place the binding plate on top of a 96-well collection plate. Place a plate sealer on top of the binding plate. Tape the sides of the two plates together to prevent an accidental spill. Centrifuge the plates at 1000 × *g* for 10 minutes.

   Note *This step is to ensure that any ethanol from the wash solution is removed prior to adding elution buffer. Ethanol contamination in the eluted PCR product can cause samples to float out of the wells of agarose gels and may result in anomalous sequencing results.*

9. Remove the binding plate and 96-well collection plate from the centrifuge. Remove the 96-well collection plate and place the binding plate on top of a fresh 96-well collection plate. Make sure both plates are correctly oriented A1 to A1. Remove the plate sealer from the binding plate. Add 50 µl of elution buffer (see *Preparation of Media and Reagents)* **directly onto the top of the fiber matrix** at the bottom of each well using a multichannel pipet. Replace the plate sealer and tape the sides of the two plates together to prevent an accidental spill.

10. Incubate the binding plate at room temperature for 5 minutes.

    Note *Maximum recovery of PCR product from each well of the binding plate depends on pH, ionic strength, volume of elution buffer added, placement of the elution buffer into the wells, and incubation time. Maximum recovery is obtained if the elution buffer is ≤10 mM Tris in concentration at pH 7–9, not less than 50 µl of elution buffer is added directly onto the fiber matrix at the bottom of each well, and incubation is performed for at least 5 minutes.*

11. Centrifuge the binding plate and 96-well collection plate together at 1000 × *g* for 5 minutes.

    Note *The purified PCR products are in the bottom of each well of the 96-well collection plate. Place a storage mat on top of the 96-well collection plate for storing the purified PCR products.*

TROUBLESHOOTING

Observation	Suggestion
Low recovery of PCR product	Verify that the PCR product has been synthesized by running a portion of the unpurified PCR product on an agarose gel
	Ensure that the 5× PCR wash buffer has been diluted to 1× PCR wash buffer by adding four volumes of 100% (v/v) ethanol to the 5× PCR wash buffer (see step 4)
	Add at least 50 µl of elution buffer to the well
	Ensure complete coverage of the membrane by dispensing the elution buffer directly onto the fiber matrix and not down the side of the well.
	Incubate the tube for a full 5 minutes after adding the elution buffer
The DNA floats out of the wells of the agarose gel	Prevent ethanol contamination by making sure that all the wash buffer is removed from the well by centrifugation before adding elution buffer

PREPARATION OF MEDIA AND REAGENTS

Elution Buffer	**5× PCR Wash Buffer**
10 mM Tris base Adjust pH to between 8.0 and 8.5 with HCl	10 mM Tris-HCl (pH 7.5) 100 mM NaCl

REFERENCE

1. Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci.* 76: 615–619.

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

- Mix an equal volume of DNA-binding solution with the aqueous portion of the PCR products
- Transfer the mixtures to a StrataPrep 96-well binding plate on a vacuum manifold
- Remove all of the liquid by vacuum; continue vacuuming for 5 minutes after the wells first appear dry
- Add 1× PCR wash buffer to the wells of the binding plate
- Remove all of the liquid by vacuum
- Discard the wash buffer and continue the vacuum for an additional 5 minutes
- Place the binding plate on a 96-well collection plate and centrifuge the plates at 1000 x g for 10 min
- Place the 96-well plate on a fresh 96-well collection plate
- Add 50 µl of elution buffer **directly onto the fiber matrix** of each well of the binding plate and incubate at room temperature for 5 minutes
- Centrifuge the two plates together at 1000 × *g* for 5 minutes; the purified PCR products are in the bottom of each well of the 96-well collection plate