Dec 15, 2016

# SpinSmart PCR Clean-up Protocol

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.gr5bv86

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**DOI:** dx.doi.org/10.17504/protocols.io.gr5bv86

**External link:** https://www.denvillescientific.com/products/spinsmart%E2%84%A2-pcr-purification-gel-extraction-columns-only--with-collection-tubes--50-per-pack

**Protocol Citation:** Denville Scientific 2016. SpinSmart PCR Clean-up Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.gr5bv86



**Protocol status:** Working

**Created:** December 15, 2016

**Last Modified:** March 24, 2018

**Protocol Integer ID:** 4637

## Abstract

The SpinSmart PCR purification and gel extraction technologies utilize a lysis buffer containing chaotropic salts that allow DNA to bind to a silica membrane. Binding buffer PCR 1 is added to a PCR reaction or agarose gel slice; the mixture is subsequently loaded directly onto SpinSmart PCR Columns.  Salts, enzymes, and other soluble components are washed away with ethanolic PCR 2 Wash buffer. Purified DNA is eluted using PCR 3 Elution buffer (5 mM Tris/HCl, pH 8.5).

## Guidelines

**Components**

SpinSmart PCR Purification and Gel Extraction Kit Contents

| Catalog Number | 50 preps<br>CM-510-50 | 250 preps<br>CM-510-250 |
|---|---|---|
| PCR 1 Binding Buffer | 2 x 25 ml | 2 x 120 ml |
| PCR 2 Wash Buffer (Concentrate)* | 2 x 6 ml | 40 ml |
| PCR 3 Elution Buffer<br>(5 mM Tris/HCl, pH 8.5) | 15 ml | 50 ml |
| SpinSmart PCR Columns (yellow ring) | 50 | 250 |
| Collection Tubes (2ml) | 50 | 250 |
| User Manual | 1 | 1 |

**Equipment and reagents to be supplied by user**

**Consumables**

96 - 100% ethanol
1.5 ml microcentrifuge tubes
Disposable pipette tips

**Equipment**

Manual pipettors
Centrifuge for microcentrifuge tubes
Heating block
Vortex mixer
Personal protection equipment (lab coat, gloves, goggles)

**The SpinSmart PCR Purification and Gel Purification Procedures**

The SpinSmart PCR purification and gel extraction technologies utilize a lysis buffer containing chaotropic salts that allow DNA to bind to a silica membrane. Binding buffer PCR 1 is added to a PCR reaction or agarose gel slice; the mixture is subsequently loaded directly onto **SpinSmart PCR Columns**. Salts, enzymes, and other soluble components are washed away with ethanolic PCR 2 Wash buffer. Purified DNA is eluted using PCR 3 Elution buffer (5 mM Tris/HCl, pH 8.5).

**SpinSmart PCR Kit Specifications**

SpinSmart PCR kits are designed for DNA purification from TAE/TBE agarose gels and for the direct purification of PCR* products.

SpinSmart PCR buffers are formulated to completely remove primers from PCR* reactions. Small double-stranded DNA fragments still remain bound and are purified with high efficiency.

SpinSmart PCR kits will effectively purify DNA fragments from detergent-rich PCR* reaction buffers.

DNA absorption to the membrane is pH-dependent. TAE standard gels or reaction mixtures with pH 6-8 should be used for best results.

Both standard and low melting agarose gels can be used.

SpinSmart PCR purified DNA fragments are ready to use in downstream applications like automated fluorescent DNA sequencing, PCR (PCR is patented by Roche Diagnostics), ligation reactions, or other types of enzymatic manipulation.

| **SpinSmart PCR Parameters** | |
|---|---|
| DNA fragments from agarose gels | 60 bp – 10 kbp |
| Elution volume | 15-50 µl |
| Binding capacity | 15 µg |
| Time/prep | 10 min for 6 preps |
| Removal of small DNA fragments and **primer-dimers** | see pages 6-7 |

**Removal of small DNA fragments and primer-dimers**

**Spin Smart PCR i**s specially formulated to remove unused, single stranded primers while effectively purifying PCR products down to 60 bp. In some cases, a PCR reaction may yield unwanted small double stranded fragments, such as primer-dimers or small PCR products resulting from unspecific annealing. SpinSmart PCR offers a simple method to remove these products that can interfere with your downstream sequencing or cloning applications.

By simply diluting PCR 1 Binding Buffer with sterile water, you can decrease the ability of small DNA fragments to bind to the membrane without compromising larger fragment recovery.  A simple dilution series should be tested, ranging from 1:1 – 1:9 (PCR 1:H2O) in order to determine the appropriate cutoff range for your reaction.  As you approach the 1:9 dilution, the larger fragment recovery will sequentially decrease as well.

*Rule of Thumb*: The smaller the fragment you wish to exclude, the less you will need to dilute the PCR 1 Binding Buffer.

**Elution procedures**

DNA should be eluted using the PCR 3 Elution Buffer.  If necessary, sterile water or other low salt elution buffers may be used, however the pH must be in the range of 7.0 – 8.5 for optimal recovery.

Typical recovery of 70-95% can be obtained with DNA fragments between 50bp -10kbp with an elution volume of 15 µl. For larger amounts of DNA (5-15 µg of DNA; from PCR* reactions > 100 µl or gel slices > 200 mg), elution with at least 50 µl of PCR 3 Elution Buffer is recommended.

Pre-warmed PCR 3 Elution Buffer can improve the yields of larger fragments (> 5-10 kbp). Add pre-warmed PCR 3 Elution Buffer (70°C) to the membrane, and incubate for 1-2 minutes, then centrifuge as directed in the standard protocol.

| DNA Recovery with SpinSmart PCR | | |
|---|---|---|
| **Fragment length** | **Elution volume** | **Recovery** |
| 65 bp | 15 µl | 85% |
| | 25 µl | 90% |
| | 50 µl | 95% |
| | 100 µl | 95% |
| 400 bp | 15 µl | 85% |
| | 25 µl | 95% |
| | 50 µl | 100% |
| | 100 µl | 100% |
| 700 bp | 15 µl | 85% |
| | 25 µl | 90% |
| | 50 µl | 95% |
| | 100 µl | 95% |
| 1500 bp | 15 µl | 85% |
| | 25 µl | 85% |
| | 50 µl | 90% |
| | 100 µl | 95% |

**Storage and preparation of solutions**

PCR 1 Wash Buffer contains chaotropic salt. Wear gloves and goggles!

SpinSmart PCR kit components should be stored at room temperature and are stable for up to one year.

The following should be prepared before starting any SpinSmart PCR purification or gel extraction protocols:

Add the indicated volume of 96-100% ethanol to PCR 2 Wash Buffer Concentrate.

| | PCR 2 Wash Buffer (Concentrate) | **50 preps** | **250 preps** |
|---|---|---|---|
| | 2 × 6 ml<br>add 24 ml 96-100% EtOH to each bottle | 40 ml<br>add 160 ml 96-100% EtOH | |

**Safety instructions – risk and safety phrases**

The following components of the SpinSmart PCR kits contain hazardous materials.
*Wear gloves and goggles and follow the safety instructions given in this section!*

| Buffer | Hazard Contents | Hazard Symbol | | Risk Phrases | Safety Phrases |
|---|---|---|---|---|---|
| PCR 1 | Guanidine thiocyanate | Xn* | Harmful by inhalation, in contact with skin and if swallowed | R 20/21/22 | S 13 |

***Risk Phrases***
*R 20/21/22 Harmful by inhalation, in contact with the skin and if swallowed*
***Safety Phrases***
*S 13 Keep away from food, drink and animal feedstuffs*

*∗ Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)*

**Troubleshooting**
For troubleshooting see the product manual:
https://www.denvillescientific.com/Files/Files/Denville/ProductDocs/CM-510-50_and_CM-510-250_manual.pdf

## Materials

MATERIALS

SpinSmart™ PCR Purification & Gel Extraction Columns Only, with Collection Tubes, 50 per pack **Denville Scientific Inc. Catalog #**CM-500-50

## Safety warnings

PCR 1 Wash Buffer contains chaotropic salt. Wear gloves and goggles!

**Safety instructions – risk and safety phrases**
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## Before start

The following should be prepared before starting any SpinSmart PCR purification or gel extraction protocols:

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|---|---|---|---|
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## Adjust DNA binding conditions

1 Mix **1 volume** of **sample** with **2 volumes** of **PCR 1 Binding Buffer** (e.g. mix 100 µl PCR reaction and 200 µl PCR 1).

Note

For sample volumes < 100 µl, adjust the volume of the reaction mix to 100 µl using PCR 1 or water.

Note

Dilutions of PCR 1 may be used for removal of primers or nonspecific products

**Spin Smart PCR is** specially formulated to remove unused, single stranded primers while effectively purifying PCR products down to 60 bp. In some cases, a PCR reaction may yield unwanted small double stranded fragments, such as primer-dimers or small PCR products resulting from unspecific annealing. SpinSmart PCR offers a simple method to remove these products that can interfere with your downstream sequencing or cloning applications.

By simply diluting PCR 1 Binding Buffer with sterile water, you can decrease the ability of small DNA fragments to bind to the membrane without compromising larger fragment recovery. A simple dilution series should be tested, ranging from 1:1 – 1:9 (PCR 1:H2O) in order to determine the appropriate cutoff range for your reaction. As you approach the 1:9 dilution, the larger fragment recovery will sequentially decrease as well.

Rule of Thumb: The smaller the fragment you wish to exclude, the less you will need to dilute the PCR 1 Binding Buffer.

## Bind DNA

2 Place a SpinSmart PCR Binding Column (yellow ring) into a Collection Tube (2 ml) and load the sample.

3 Centrifuge for **1 min** at **11,000** x g.

00:01:00

4 Discard flow-through and place the SpinSmart PCR Binding Column back into the Collection Tube.

## Wash silica membrane

5 Add **600 µl PCR 2 Wash Buffer**.

6 Centrifuge for **1 min at 11,000 x g.**
00:01:00

7 Discard flow-through and place the SpinSmart PCR Binding Column back into the Collection Tube.

Note

Optional: To prevent salt carryover for sensitive procedures, add an additional 200 µl PCR 2 Wash Buffer and repeat wash steps 5-7.

## Dry silica membrane

8 Centrifuge for **2 min** at **11,000 x g** to remove PCR 2 Wash Buffer. The tip of the spin column should not come in contact with the flow-through while removing it from the centrifuge and the Collection Tube.
00:02:00

Note

Residual ethanol from Wash Buffer PCR 2 can inhibit subsequent reactions and must be removed in this step. Complete EtOH removal can be achieved by incubation of SpinSmart PCR Columns for 2-5 min at 70°C prior to elution.

## DNA Elution

9 Place the SpinSmart PCR Binding Column into a clean 1.5 ml microcentrifuge tube (not provided)

10 Add **15 - 50 µl PCR 3 Elution Buffer** and incubate at **room temperature** for **1 min to increase** the yield of eluted DNA.
00:01:00

11 Centrifuge for **1 min at 11,000 x g**.
00:01:00

Note

Prewarmed PCR 3 Elution Buffer (70°C) can be used to increase the yield of larger fragments (> 5-10 kbp).