SpinPrep™ PCR Clean-Up Kit

About the Kit

Introductory SpinPrep PCR Clean-Up Kit	20 rxn	70975-3
SpinPrep PCR Clean-Up Kit	100 rxn	70976-3

Description

The SpinPrep PCR Clean-Up Kit is designed for the rapid purification of DNA amplified in PCR. The 10-minute procedure involves addition of a binding buffer followed by adsorption of the DNA to a silica membrane in a spin column format. Following a wash step, the DNA is eluted in low-salt buffer. This kit removes DNA polymerases, dNTPs, salts, and > 99% of primers so that they do not interfere with downstream applications such as cloning, sequencing, or labeling. Each SpinPrep PCR Filter has a total DNA binding capacity of up to 6 µg. PCR products from 100 to > 12,000 bp can be cleaned up, with recoveries of amplified DNA in the range of 60–90% under standard conditions. The SpinPrep PCR Clean-up Kit includes 1.5-ml Eluate Receiver Tubes with caps that can be sealed during the elution step, preventing evaporation.

Components

- 18 or 82 ml SpinPrep Bind Buffer (A)
- 27 ml SpinPrep Wash Buffer (B)
- 10 ml SpinPrep Elute Buffer (C)
- 20 or 100 SpinPrep PCR Filters
- 20 or 100 Receiver Tubes (2ml)
- 20 or 100 Eluate Receiver Tubes (1.5 ml)

Storage

Store all components at room temperature.

Required equipment and reagents

- 100% ethanol
- microcentrifuge
- 70°C water bath or heat block

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
Freefone 0800 622935
Toll Free 1800 409445
customer.service@
merckbiosciences.co.uk

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

A Brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany
www.novagen.com

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General Considerations

Individual spin units have a binding capacity of up to 6 µg DNA. The protocol is designed to process 50–150 µl standard PCR reactions. Yields will vary depending on the starting material, with a typical recovery of 60–90% of the amplified DNA. Complete elution and the highest recovery efficiencies of the captured PCR products are achieved by performing two sequential elutions.

The SpinPrep™ PCR Filters will efficiently bind and elute PCR fragments from 150 bp to 12,000 bp. As the DNA size decreases below 150 bp, effective binding and elution decreases. DNA fragments between 50 and 150 bp in size may also be purified but recovery efficiencies will be significantly lower. Up to 95% of 50 bp fragments and up to 99% of DNA fragments less than or equal to 20 bp will be removed.

Note: *Amplification reaction conditions can dramatically affect amplified DNA yield and quality. Variables affecting yield and quality include; template, primer, dNTP, polymerase and free Mg++ concentrations, template complexity, primer design, buffer components, polymerase type, cycle number and reaction volume. Novagen recommends starting with a standard reaction, and optimizing PCR conditions to achieve the desired yield and fidelity of the amplification product.*

PCR Clean-up Procedure

Note: ***Prior to the first use of the kit, reconstitute the SpinPrep Wash Buffer by adding 63 ml of 100% ethanol directly to the supplied bottle.*** *After ethanol has been added, mark the bottle label as a reminder.*

1. Before beginning, place the required volume (50–100 µl per prep) of SpinPrep Elution Buffer (C) at 70°C to warm up.
2. Transfer the completed PCR reaction, leaving the oil overlay in the reaction tube, to a clean 1.5-ml microcentrifuge tube. Add 4 vol (400 µl per 100 µl PCR reaction) SpinPrep Bind Buffer (A) and vortex to mix completely.

Note: *Carryover of small amounts of the oil overlay will not affect the purification.*

3. Place a SpinPrep PCR Filter in a 2-ml Receiver Tube, transfer the mixture from step 2 to the spin unit and centrifuge the spin unit at ≥ 10,000 × g for 1 min.
4. Remove the filter unit from the Receiver Tube and discard the flow through.
5. Replace the filter unit into the 2-ml Receiver Tube and add 400 µl SpinPrep Bind Buffer (A). Centrifuge the spin unit at ≥ 10,000 × g for 1 min.
6. Remove the filter unit from the Receiver Tube and discard the wash.
7. Replace the filter unit into the 2-ml Receiver Tube and add 500 µl reconstituted SpinPrep Wash Buffer (B). Centrifuge the spin unit at ≥ 10,000 × g for 1 min.
8. Remove the filter unit from the receiver tube and discard the wash.
9. Replace the filter unit into the 2-ml Receiver Tube and centrifuge the spin unit at ≥ 10,000 × g for 2 min to remove residual SpinPrep Wash Buffer.
10. Transfer the SpinPrep Filter to the provided 1.5-ml Eluate Receiver Tube.
11. Pipet 50 µl of prewarmed (70°C) SpinPrep Elute Buffer (C) onto the SpinPrep Filter membrane. Close the cap of the receiver tube and incubate for 3 min at room temperature.
12. Immediately centrifuge for 1 min to collect the eluted PCR product.
13. For maximum DNA yield, perform a second elution by repeating steps 11–12.

Troubleshooting guide

The expected recovery of amplified DNA is typically 60–90% of the input material. Amplification reaction conditions can dramatically affect the amount and quality of amplified DNA in the PCR reaction. Other protocol-dependent factors that can affect recovery of amplified DNA are outlined in the table below.

Problem	Probable Cause	Solution
Low DNA yield	Incomplete DNA elution	Work quickly to transfer the heated buffer to the membrane. Higher DNA concentrations can be obtained by using 30 µl or less of the Elute Buffer. DNA can also be eluted with water or 10 mM Tris-HCl pH 8.0. DNA samples eluted without EDTA should be stored at –20°C to avoid bacterial growth.
	Incomplete mixing of the PCR reaction and the SpinPrep™ Bind Buffer	Vortex the SpinPrep Bind Buffer and PCR reaction mixture prior to transferring it to the spin unit.
	Target DNA size is below capture cutoff	The SpinPrep PCR Filters will bind DNA greater than 150 bp most efficiently and 100 bp moderately efficiently.
	pH of binding reaction too high	PCR reactions utilizing strongly alkaline conditions may increase the pH of the binding reaction. Add 2.5 µl 1M Tris-HCl pH 7.5 to a 100 µl PCR reaction prior to addition of the SpinPrep Bind Buffer (A).
Co-purification of small (< 50 bp) fragments	pH of binding reaction to low	PCR reactions that drive the binding pH down may cause copurification of PCR artifacts. Add 2.5 µl of 1 M Tris-HCl 7.5 to a 100 µl PCR reaction prior to addition of the SpinPrep Bind Buffer (A).
DNA floats out of well during agarose gel loading	Residual ethanol in eluted DNA	The SpinPrep Filter must be completely free of residual SpinPrep Wash Buffer (C) prior to DNA elution. Make sure the spin (step 9) is for a full 2 min. Residual ethanol can also be removed by vacuum drying before elution or by precipitating the eluted DNA with ethanol and drying to completion.

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