SpinPrep™ Gel DNA Kit

SpinPrep Gel DNA Kit 100 rxn 70852-3

About the Kit

Description

The SpinPrep Gel DNA Kit efficiently extracts DNA fragments of 150 bp to >12,000 bp in size from agarose gels. The method is based on DNA binding to silica at neutral to slightly acidic pH and high salt and is compatible with the two most popular agarose gel electrophoresis buffer systems: Tris-acetate, EDTA (TAE) and Tris-borate, EDTA (TBE). The kit includes SpinPrep filters with silica membranes, receiver tubes, and eluate receiver tubes with caps that can be sealed during elution. The protocol does not require extraction with phenol or ethanol precipitation steps. Reagents are included for melting the agarose gel slice and binding DNA to the silica membrane; washing to remove protein, salts, and ethidium bromide; and elution from the membrane. The entire procedure requires less than 30 minutes and the isolated DNA is suitable for restriction analysis, PCR, sequencing, transformation, and general cloning work. Each spin unit has a total DNA binding capacity of up to 20 µg.

Components

- 27 ml SpinPrep Wash Buffer (B)
- 10 ml SpinPrep Elute Buffer (C)
- 100 SpinPrep Filters
- 100 Receiver Tubes (2 ml)
- 100 Eluate Receiver Tubes (1.5 ml)
- 5 × 24 ml SpinPrep GelMelt™ Solution (A)

Storage

Store all components at room temperature.

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Extraction of DNA from agarose gel

The following protocol describes extraction and purification of DNA from bands on agarose gels. It is not necessary to use low melt agarose. The DNA should be electrophoresed on an agarose gel, stained, and bands visualized by long wavelength UV. The volume of GelMelt™ Solution (A) provided in the 100 rxn kit is sufficient for 100 slices averaging 150 mg in size. Generally, use one SpinPrep™ unit per band to be extracted. If isolating preparative quantities of DNA fragments in excess of 20 µg, two or more spin units will be required.

Note: *Protect eyes and skin from UV exposure. Wear UV blocking safety glasses and gloves. Work quickly to limit exposure time to UV light.*

Preparation

Prior to the first use of the kit, add 63 ml of 100% ethanol to the 27 ml SpinPrep Wash Buffer (B). After ethanol has been added, mark the check box on the bottle label as a reminder.

Assemble necessary items: Stained DNA agarose gel, UV source and protective wear, scalpels, 1.5-ml microfuge tubes, SpinPrep GelMelt Solution (A), SpinPrep Wash Buffer (B) Plus Ethanol, SpinPrep Elute Buffer (C), an appropriate number of SpinPrep units (filter, receiver tube, eluate receiver tube). Place the required volume (50 µl per prep) of SpinPrep Elute Buffer (C) at 50°C to pre-warm.

Weigh 1.5-ml tubes to be used for melting gel slices and note the weight of each tube (± 0.01 g).

Procedure

1. Place gel on a long-wavelength UV light box. Using a clean scalpel or razor blade, excise the desired DNA band(s) from the gel. Look at the gel slice from several angles and trim off excess agarose.

Note: *Extended UV exposure can cause damage to the DNA. Use a long-wavelength UV source and work quickly to minimize DNA damage. To prevent scratches on the transilluminator filter, cut gels on a thick sheet of UV transparent plastic.*

2. Place gel slice in the pre-weighed 1.5-ml tube and weigh. Record the weight of gel slice.
3. For gels up to 2% agarose, add 300 µl SpinPrep GelMelt Solution (A) per 100 mg of gel slice. For gels of greater than 2% agarose or slices greater than 300 mg, add 600 µl SpinPrep GelMelt Solution (A) per 100 mg of gel slice.
4. Close tube and vortex briefly. Transfer tube to 50°C bath or heat block. Incubate for 10 min to dissolve the gel slice. Confirm that gel slice has melted by inspecting tube contents for viscous lumps. If necessary, incubate longer until gel slice has fully melted.
5. Place a SpinPrep Filter into a 2-ml receiver tube. Transfer up to 700 µl of the dissolved gel solution to SpinPrep Filter. Centrifuge at top speed in a microcentrifuge for 30 s. Multiple loads of the melted gel slice should be applied if the total volume exceeds 700 µl. Discard flow-through after each spin and pipet additional solution into SpinPrep Filter. Repeat until entire volume of melted gel solution has been passed through SpinPrep Filter.
6. Discard flow-through from the receiver tube. Return the SpinPrep Filter to the Receiver Tube and add 400 µl fresh SpinPrep GelMelt Solution (A). Centrifuge 30 s and discard flow-through.
7. Add 650 µl SpinPrep Wash Buffer (B) Plus Ethanol and centrifuge 30 s.
8. Discard flow-through and centrifuge an additional 2 min to remove residual SpinPrep Wash Buffer (B) Plus Ethanol.
9. Transfer SpinPrep Filter to the provided 1.5-ml Eluate Receiver Tube.
10. Pipet 50 µl prewarmed (50°C) SpinPrep Elute Buffer (C) onto the SpinPrep Filter membrane. Close receiver tube cap and incubate 3 min at 50°C.
11. Immediately centrifuge 1 min to collect the eluted DNA into the Eluate Receiver Tube. (For maximum DNA yield, perform a second elution by repeating steps 10–11.)
12. Discard SpinPrep Filter. Cap Eluate Receiver Tube and store DNA at 4°C or –20°C.

Troubleshooting guide

The described protocol results in recoveries that range from 50–90%, depending on the size and the amount of the DNA to be extracted. The protocol is robust and has been used successfully with a broad range of DNA fragment sizes (150 – 12,000 bp) and agarose gel percentages. When low yield does occur, it can usually be traced to problems outlined in the table below.

Problem	Probable Cause	Solution
Low DNA yield	Insufficient amount of SpinPrep™ GelMelt™ Solution added to gel slice (Step 3)	Repeat DNA isolation with carefully weighed gel slice. Use 300 µl SpinPrep GelMelt Solution per 100 mg of gel slice. For gel slices of greater than 2% agarose use 600 µl SpinPrep GelMelt Solution per 100 mg gel slice.
	Incomplete solubilization of the gel slice (Step 4)	Larger gel slices and gel slices of higher agarose take longer to dissolve. In these cases use up to 600 µl SpinPrep GelMelt Solution per 100 mg of gel and increase the incubation time to 20–30 min. Larger gel slices can be cut into small pieces, which will enhance solubilization.
	Incomplete DNA binding (Step 5)	Binding of the DNA to the silica membrane occurs at neutral to slightly acidic pH. Depending on the electrophoresis gel buffer system, binding may be enhanced by adding 20 µl of 1 M Tris-HCl, pH 7.0 in Step 5.
	Incomplete DNA elution	Preheating the SpinPrep Elute Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to 65–70°C can improve the elution efficiency, especially when DNA fragments are larger than 5 kbp. If incomplete elution is the cause of the low yield, a second elution step (repeat of Step 12) can be performed to increase the yield. Higher DNA concentrations can be obtained by using 30 µl or less preheated SpinPrep Elute Buffer. DNA can also be eluted in water or 10 mM Tris-HCl pH 8.0. DNA samples eluted in buffers without EDTA should be stored at –20°C to avoid bacterial growth.
DNA floats out of well during agarose gel loading.	Residual ethanol in eluted DNA	The spin unit must be completely free of residual SpinPrep Wash Buffer prior to DNA elution to avoid ethanol carry-over. Make sure spin (Step 10) is for at least 2 min. Residual ethanol can be removed by vacuum evaporation or by ethanol precipitation and drying to completion.