SpinPrep™ Plasmid Kit

SpinPrep Plasmid Kit 100 rxn 70851-3

About the Kit

Description

The SpinPrep™ Plasmid Kit is designed for rapid miniprep isolation of plasmid DNA from 1.5–3 ml bacterial cultures. The method combines standard alkaline lysis with DNA binding and purification on silica membranes. The protocol does not require organic extraction or alcohol precipitation steps, and takes less than 30 minutes. Isolated DNA is suitable for restriction analysis, PCR, sequencing, transformation, and general cloning work. Each SpinPrep Filter can bind up to 20 µg plasmid DNA. Sufficient reagents are included for 100 plasmid purifications.

Components

- 0.1 ml RNase A Solution
- 10 ml Bacterial Resuspension Buffer (1)
- 20 ml Bacterial Lysis Buffer (2)
- 40 ml SpinPrep™ Neutralization Buffer(A)
- 27 ml SpinPrep Wash Buffer (B)
- 10 ml SpinPrep Elute Buffer (C)
- 100 SpinPrep Filters
- 100 SpinPrep Receiver Tubes
- 100 SpinPrep Eluate Receiver Tubes

Storage

Store all components at room temperature. After addition of RNase A, Bacterial Resuspension Buffer (1) should be stored at 4°C.

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Protocol

This protocol is designed to process 1.5 ml culture for high copy number plasmids, or 3 ml for low copy number plasmids. Typical yields per ml of culture are 5–10 µg DNA for high copy number plasmids or 0.25–1 µg for low copy number plasmids. Higher yields of low copy number plasmid may be obtained by processing up to 30 ml culture through one SpinPrep™ Filter. In this case, pellet the culture using multiple tubes in parallel (pellet 1.5 ml culture in each of 10 tubes, aspirate media, and pellet an additional set of 1.5 ml aliquots).

Although most host strains can be used successfully with the SpinPrep Plasmid Kit, plasmid DNA quality can be host-dependant. The SpinPrep Plasmid Kit yields high-quality plasmid from Novagen's NovaBlue strain. Quality is optimal in strains such as NovaBlue bearing mutations in *endA* (eliminating endonuclease I activity) and *recA* (eliminating general recombination and inhibiting formation of plasmid multimers).

Culture conditions

Culture conditions can dramatically affect plasmid yield and quality. Culturing in LB broth is recommended (see recipe below). Rich media such as Terrific Broth may increase cell mass, but yield and quality of plasmid may suffer, as greater levels of cellular and media components interfere with plasmid binding to the silica membrane.

Preparation of LB broth: Dissolve 10 g tryptone, 5 g yeast extract and 10 g NaCl in ~900 ml deionized water. Adjust pH to 7.5 with 1 N NaOH. Adjust volume to 1 liter with deionized water and autoclave. Cool to ~60°C before adding appropriate antibiotic.

Cultures should always be grown in presence of appropriate antibiotic to maintain plasmid selection. Particular care should be taken with the commonly-used ampicillin resistance marker β-lactamase. Cultured cells secrete β-lactamase into the medium, leading to rapid breakdown of antibiotic. Ampicillin degradation is enhanced during the late stages of cell growth, when culture pH drops. Carbenicillin (Cat. No. 69101-3), which is less acid-labile than ampicillin, may be substituted.

Cultures for plasmid isolation are prepared by inoculating 2–5 ml antibiotic-containing LB medium with a well-isolated colony from a freshly streaked, antibiotic-containing LB agar plate. Shake liquid culture at 300 rpm for 12–16 h at 37°C.

Note: *Prior to first use of the kit, add 63 ml 100% ethanol to the 27 ml of SpinPrep Wash Buffer (B). Mark the bottle label to indicate that ethanol has been added.*

Add the supplied 0.1 ml RNase A Solution to the 10 ml Bacterial Resuspension Buffer. Mark the bottle label to indicate that RNase A has been added. Store this solution at 4°C.

1. Before beginning, pre-warm SpinPrep Elution Buffer (C) (50 µl needed for each sample) at 50°C.
2. Transfer 1.5 ml overnight culture to a clean 1.5 ml microcentrifuge tube and centrifuge at 10,000 rpm (7,400 × *g*) for 1 min. Remove culture supernatant without disturbing pelleted cells. If processing 3 ml low copy number plasmid culture, repeat this step by pelleting another 1.5 ml of culture in the same tube, and double all volumes in subsequent steps (except for the elution step). If processing larger volumes, divide culture into 3 ml aliquots and pellet aliquots using multiple centrifuge tubes in parallel (3 ml per tube). Up to 30 ml culture can be processed through a single SpinPrep Filter.
3. Resuspend cell pellet in 100 µl Bacterial Resuspension Buffer (1). Vortex gently to completely resuspend. Complete resuspension of the pellet is critical.
4. Add 200 µl Bacterial Lysis Buffer (2). Mix gently by inverting sample 6 times. Do not vortex, as doing so can cause shearing of genomic DNA.
5. Incubate tube for 5 min at room temperature.
6. Carefully open tube and add 400 µl SpinPrep Neutralization Buffer (A). Mix gently by inverting. Do not vortex.
7. Centrifuge for 10 min at top speed in a microcentrifuge.

8. Place SpinPrep™ Filter in 2 ml SpinPrep Receiver Tube. Transfer up to 650 µl of the supernatant from Step 7 to filter unit. When removing supernatant, do not disturb white pellet. Centrifuge at top speed in a microcentrifuge for 30 sec. If processing larger volumes, repeat with another 650 µl supernatant in the same tube.
9. Discard filtrate. Add 650 µl reconstituted SpinPrep Wash Buffer (B) (containing ethanol) to the SpinPrep Filter. Centrifuge for 30 sec.
10. Discard filtrate. Centrifuge for 2 min to remove residual SpinPrep Wash Buffer.
11. Transfer SpinPrep Filter to the provided 1.5 ml Eluate Receiver Tube.
12. Pipet 50 µl prewarmed (50°C) SpinPrep Elute Buffer (C) onto each SpinPrep Filter membrane. Close cap of Receiver Tube. Incubate for 3 min at 50°C.
13. Immediately centrifuge for 1 min to collect eluted plasmid DNA.
14. For maximum DNA yield, perform a second elution by repeating steps 12–13. Discard SpinPrep Filter after use.

Determination of Plasmid Yield and Purity

UV absorbance measurements

DNA yield can be determined spectrophotometrically by measuring A_{260} of a diluted sample. For a 1 cm path length cuvette, A_{260} 1.0 = 50 µg/ml dsDNA. For optimal accuracy, target absorbance for UV spectrophotometer readings should be between 0.1–0.5. Therefore, sample plasmid DNA should be diluted 1:40 to 1:200 in deionized water prior to measuring A_{260}. To obtain the concentration of plasmid DNA in µg/ml, multiply A_{260} by the dilution and the extinction coefficient (50 µg/ml/1.0 A_{260}).

Results of A_{260} readings are accurate only in absence of significant contamination by RNA and other UV-absorbing materials. Critical samples should also be analyzed by agarose gel electrophoresis. The A_{260}/A_{280} ratio can indicate sample purity. Nucleic acids have an average absorbance maxima of ~260 nm, whereas proteins (assuming normal distribution of aromatic residues) have an average absorbance maximum of 280 nm. Pure DNA and typical SpinPrepplasmid samples have A_{260}/A_{280} ratio of 1.75–1.95. Preparations contaminated with protein have significantly lower ratios (1.3–1.5), while higher ratios (≥2.0) may indicate RNA contamination.

Troubleshooting guide

Plasmid yield can be affected by a number of factors including host genotype, plasmid copy number, plasmid size, and specific DNA sequences. Other factors that can affect yield are outlined below.

Problem	Probable Cause	Solution
Low DNA yield	Supernatant not completely removed prior to resuspension and lysis	Residual media can shift pH during the subsequent lysis step, resulting in inefficient lysis. Repeat procedure, ensuring full removal of liquid from cell pellet before proceeding.
	Incomplete resuspension of pellet in Step 3	Incomplete resuspension can cause inefficient lysis and lower plasmid yields. Repeat procedure, being careful to fully resuspend the cell pellet.
	Plasmid is low copy number	For low copy number plasmids, higher yield can be obtained by increasing culture volume processed on each SpinPrep™ Filter. Do not process more than 30 ml culture through one SpinPrep Filter.
	Incomplete DNA elution	Elution efficiency can be improved by preheating SpinPrep Elute Buffer (C) to 65–70°C. Higher DNA concentrations can be obtained by using 30 µl or less pre-warmed SpinPrep Elute Buffer. DNA can also be eluted with water or 10 mM Tris HCl pH 8.0. DNA samples eluted in absence of EDTA should be stored at –20°C.
Chromosomal DNA contamination	Incomplete precipitation of chromosomal DNA during Step 5, or shearing during handling	Chromosomal DNA normally precipitates upon addition of SpinPrep Neutralization Buffer (A) and centrifugation. Chromosomal DNA contamination can be reduced or eliminated by avoiding vortexing during Steps 2 through 5. Efficient pelleting requires a full 10 min centrifugation in Step 6.
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 centrifugation (Step 10) is conducted for a full 2 min. Residual ethanol can also be removed by vacuum drying or by precipitating eluted DNA with ethanol and drying to completion.

Related Products

Products	Size	Cat. No.
Carbenicillin	5 g	69101-3
Chloramphenicol	25 g	220551
Kanamycin Sulfate	5 g	420311
Tetracycline Hydrochloride	25 g	58346
Perfect DNA™ Markers, 0.5–12 kbp	100 lanes	69002-3
Perfect DNA Markers, 0.1–12 kbp	100 lanes	70087-3
Perfect DNA Markers, 0.05–10 kbp	100 lanes	70540-3
6X DNA Gel Loading Buffer	1 ml	69046-3
Introductory Mobius™ 1000 Plasmid Kit	2 rxn	70854-3
Mobius 1000 Plasmid Kit	10 rxn	70853-3
	25 rxn	70853-4
Introductory UltraMobius™ 1000 Plasmid Kit	2 rxn	70907-3
UltraMobius 1000 Plasmid Kit	10 rxn	70906-3
	25 rxn	70906-4
Mobius Buffer Kit	10 rxn	70855-3
Mobius 1000 Columns	10 rxn	70849-3
	25 rxn	70849-4
ClearSpin™ Filters	pkg/25	70848-3

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