Invitrogen™
life technologies

Instruction Manual

S.N.A.P.™ MidiPrep Kit

A Simple Nucleic Acid Prep

Catalog no. K1910-01

Version F
041702
25-0192

Overview

Introduction

The S.N.A.P.™ MidiPrep Kit allows isolation of highly pure plasmid DNA that is suitable for transfection, manual or automated sequencing, PCR, restriction mapping, ligation, and transformation. The S.N.A.P.™ resin is supplied in a column and preferentially binds supercoiled plasmid DNA.

Storage

Store the kit at room temperature, except for the following components which are stored at +4°C after opening the kit:

- Resuspension Buffer
- Precipitation Salt
- RNase A

Contents

The following items are included in the S.N.A.P.™ MidiPrep Kit. Sufficient reagents are included for 20 reactions.
Note: Solutions are not interchangeable with the S.N.A.P.™ Miniprep Kit.

Item	Composition	Quantity
S.N.A.P.™ MidiPrep Column A (Filtering) Column B (Binding)	--	 20 20
Resuspension Buffer	50 mM Tris-HCl, pH 8.0, 10 mM EDTA	80 ml
RNaseA, lyophilized	--	6 mg
Lysis Buffer	0.2 M NaOH, 1% SDS	80 ml
Precipitation Salt	3 M Potassium acetate, pH 5.2	80 ml
Binding Buffer	7.5 M Guanidine-HCl	2 x 120 ml
Wash Buffer	5 M Guanidine-HCl, 50 mM MOPS, pH 7.0	100 ml
4X Final Wash	400 mM NaCl	80 ml

The Binding Buffer and Wash Buffer contain a chaotropic salt. Use gloves and protective eye wear when handling these solutions.

Using the S.N.A.P.™ MidiPrep Kit

Experimental Outline

The procedure requires the following steps:

1. Lyse cells using the Lysis Buffer
2. Precipitate protein and genomic DNA
3. Filter the lysate/precipitate through Column A
4. Add Binding Buffer to the flowthrough and apply to Column B
5. Wash the bound plasmid and dry the resin by centrifugation
6. Elute the plasmid DNA with sterile water or TE buffer.

User Supplied Reagents

- 240 ml 95% ethanol for dilution of 4X Final Wash
- 500 ml sterilized bottle for dilution of 4X Final Wash
- Sterile 50 ml conical tubes (Falcon, Sarstedt, or Corning)
- Centrifuge with rotor and rotor adapters for 50 ml conical tubes
- Sterile water or TE

Before Starting

1. Resuspend the entire contents of the RNase A tube (6 mg) in 1 ml of Resuspension Buffer. Add the resulting solution to the remaining Resuspension Buffer. Resuspension Buffer containing RNase A should now be stored at +4°C.
2. Add the 80 ml of 4X Final Wash to 240 ml of 95% ethanol to make 320 ml of 1X Final Wash. Store in a 500 ml sterilized bottle.
3. Check the Lysis Buffer for a white precipitate. If present, place the buffer in a 37°C water bath for 5 minutes or until the solution clears.
4. Use bacterial cell cultures that are 1 to 2 x 10^9 cells/ml (OD_{600} < 3.0). For cultures >3 x 10^9 cells/ml, use 50 ml or less of culture per column.

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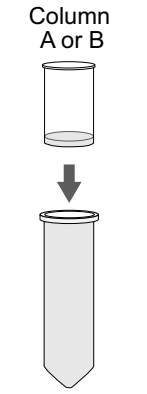
Lysis and Removal of Precipitate



You will need four 50 ml conical tubes for each plasmid preparation. All centrifugations are at room temperature except as noted.

1. Centrifuge 10-100 ml of an overnight bacterial culture at 4000 x g for 5-10 minutes at +4°C to pellet the cells. Pour off the medium (do not aspirate medium).
2. Resuspend the cell pellet in 4 ml of Resuspension Buffer by vortexing or gently pipetting up and down.
3. Add 4 ml of Lysis Solution and mix by inverting gently 5-6 times. Incubate for 3 minutes at room temperature.
4. Add 4 ml of Precipitation Salt and invert gently 6 to 8 times. Incubate5 minutes in ice. Invert tubes twice during incubation to ensure even formation of precipitate within the solution. Do not centrifuge.
5. During the incubation, label two 50 ml conical tubes "A" and "B". Place one S.N.A.P.™ MidiPrep Column A (Filtering) inside the 50 ml conical tube labeled "A", and one S.N.A.P.™ MidiPrep Column B (Binding) inside the 50 ml conical tube labeled "B" (see figure).
6. After incubation, transfer the solution from Step 4 onto Column A and centrifuge for 5 minutes at 3,000 x g. Discard Column A. Save the filtrate containing the plasmid DNA.

Plasmid Binding

1. To the filtrate add 12 ml of Binding Buffer and mix by gently inverting twice. Transfer the solution to Column B inserted in a 50 ml conical tube.
2. Centrifuge for 2 minutes at 1,000 x g. The plasmid DNA is now bound to Column B. Discard the flowthrough.
3. Add 5 ml of Wash Buffer and centrifuge for 1 minute at 2,000 x g. Discard flowthrough.
4. Add 5 ml of 1X Final Wash Buffer and centrifuge for 2 minutes at 2,000 x g.
5. Add 10 ml of 1X Final Wash Buffer and centrifuge for 2 minutes at 2,000 x g. Discard flowthrough.
6. Centrifuge Column B at >4,000 x g for 5 minutes to dry the resin.

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Plasmid Elution

1. To elute the plasmid DNA, transfer Column B to a new, sterile 50 ml conical tube and add 750 µl of sterile water or TE buffer directly to column resin.
2. Incubate for 3 minutes at room temperature. **Note:** Do not elute in less than 750 µl. Plasmid recovery will decrease.
3. Centrifuge for 5 minutes at >4,000 x g. The plasmid DNA is now eluted from Column B.
4. Remove and discard the column.

Determining DNA Yield

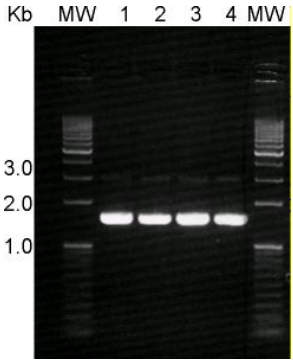
The plasmid DNA is now pure and ready for all molecular biology applications. To determine the plasmid DNA yield and concentration, use a spectrophotometer and read the absorbance at 260 nm. When using a spectrophotometer, zero the machine first with water or TE at 260 nm. Use 5-10 µl of the plasmid DNA sample to determine concentration. Use the formula give below:

$$[DNA] = (A_{260})\ (50°g/ml)\ X\ D$$

where D is the dilution factor. Determine the yield by multiplying the concentration by the volume of DNA.

Typical Yields

Maximum yields range from 10 to 200 µg DNA total, depending on the copy number of the plasmid and the volume of culture. Concentration of DNA will range from 10 to 275 µg/ml. It may be necessary to ethanol precipitate the DNA to concentrate it if you are using small culture volumes. An example of plasmid DNA isolated using the S.N.A.P.™ Kit is shown below.



Legend for the gel:

Plasmid DNA was isolated from four 50 ml TOP10F′ overnight cultures and electrophoresed on a 0.8% agarose gel.

Lanes 1-4: 500 ng of plasmid purified with the S.N.A.P.™ MidiPrep Kit

Troubleshooting

Trouble-shooting

Review the information provided in the table below to troubleshoot your experiments.

Problem	Cause	Solution
Low yield	Incomplete lysis or too much cell lysate has clogged the column	Decrease cell culture volume used. Use up to 100 ml of an overnight culture, density 2 x 10^9 cells/ml (OD_{600} < 3.0 per ml). For more dense cultures, decrease volume of culture.
		Decrease cell culture density. Use LB medium instead of TB (Terrific Broth).
	Genomic DNA present	Perform Steps 3-5 (page 5) with gentle mixing to avoid damaging DNA. Genomic DNA must be intact to be efficiently removed.
Plasmid resistant to restriction enzyme digestion	Denatured, supercoiled plasmid present (migrates below native, supercoiled plasmid)	Do not incubate longer than 3 minutes in Lysis Buffer (Step 3, page 5).

Technical Service

Contact Us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

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MSDS Requests

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1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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Technical Service, Continued

Emergency Information

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Accessory Products

Additional Products

The following products are available separately from Invitrogen.

Product	Quantity	Catalog no.
Lipofectamine™ 2000	0.75 ml	11668-027
	1.5 ml	11668-019
Lipfectamine™ Reagent	1 ml	18324-012
Lipofectin™ Reagent	1 ml	18292-011
Calcium Phosphate Transfection Kit	75 reactions	K2780-01
Plus™ Reagent	0.85 ml	11514-015
SequenceR$_x$ Enhancer System	25 reactions	12237-012

Product Qualification

Each component of the S.N.A.P.™ MidiPrep is lot qualified for maximum performance in accordance with the S.N.A.P.™ MidiPrep protocol. A sample of 4 columns from each lot is qualified in the following manner:

Binding Capacity

300 µg of pre-purified control plasmid is applied to the column and eluted according to the protocol. The quantity of plasmid DNA eluted from the column must be >200 µg.

Kit Performance

50 ml of control plasmid is grown in TOP10F′ cells and plasmid DNA is isolated using the S.N.A.P.™ MidiPrep Kit. Plasmid DNA is qualified in the following manner:

- **Visual inspection**
  Four samples are run on 0.8% agarose gel for a visual inspection. Each isolated plasmid must show supercoiled plasmid DNA with no RNA contamination.
- **Restriction Digest**
  Four 500 ng plasmid DNA samples are digested with *Apa*I restriction enzyme for 90 minutes. Restriction digest is run on a 0.8% agarose gel and must be >99% complete.
- **Endonuclease Activity**
  Four 500 ng plasmid DNA samples are incubated with 10 mM Mg^{2+} for 4 hours and then run on a 0.8% agarose gel. Each sample must show no endonuclease contamination of supercoiled DNA present.

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