S.N.A.P.™

Miniprep Kit

A Simple Nucleic Acid Prep

Version F

Catalog nos.
K1900-01
K1900-05

Invitrogen™
life technologies

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Overview

Introduction

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

The binding and washing steps can be performed by either gravity flow or centrifugation. If using centrifugation, the procedure will take 25 minutes versus 45 minutes by gravity flow. The yield and quality of DNA will be the same.

Kit Contents

The following items are included in the S.N.A.P.™ Miniprep Kit.

- 100 S.N.A.P.™ Miniprep Columns
- 100 2 ml Collection Tubes
- Resuspension Buffer (15 ml)
- RNase A, lyophilized (1.55 mg)
- Lysis Buffer (15 ml)
- Precipitation Salt (15 ml)
- Binding Buffer (60 ml)
- Wash Buffer (50 ml)
- 4X Final Wash (25 ml)
- S.N.A.P.™ laminated manual

User Supplied

- 75 ml 95% ethanol for dilution of 4X Final Wash
- 100 ml sterilized bottle or tube for dilution of 4X Final Wash
- Sterile 1.5 ml microcentrifuge tubes (3 per miniprep)
- Microcentrifuge
- Sterile water or TE

Storage

This product should be stored at room temperature, except for the following components which should be stored at +4°C:

- Resuspension Buffer
- Precipitation Salt
- RNase A

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

Before Starting

1. Resuspend the entire contents of RNase A (1.55 mg) in 200 µl of **Resuspension Buffer** and add to the remaining **Resuspension Buffer. Resuspension Buffer** should now be stored **at +4°C**.
2. Add the 25 ml of **4X Final Wash** to 75 ml of 95% ethanol to make 100 ml of **1X Final Wash.**
3. Check the **Lysis Buffer** for a white precipitate. If present, place the buffer in a 37°C water bath for 5 minutes until the solution clears.

Procedure

Lysis and Precipitation

1. Centrifuge 1-3 ml of an overnight culture to pellet the cells. Use cell cultures that are 1 to 1.5 x 10^9 cells/ml. For low copy number plasmids, refer to the **Troubleshooting** section on the following page.
2. Resuspend the cell pellet in 150 µl of **Resuspension Buffer** by vortexing or gently pipetting up and down.
3. Add 150 µl of **Lysis Buffer** and mix gently by inverting the tube 5-6 times. Incubate for 3 minutes at room temperature.
4. Add 150 µl of ice-cold **Precipitation Salt** and invert 6-8 times to ensure thorough mixing of all components.
5. Centrifuge in a microcentrifuge at room temperature at 14,000 x g for 5 minutes.
   While waiting for the centrifuge, place the S.N.A.P.™ Miniprep Column (A) inside the 2 ml Collection Tube provided (B).

Plasmid Binding

6. Pipette the supernatant into a sterile microcentrifuge tube. Discard the gelatinous pellet.
7. Add 600 µl of **Binding Buffer** and mix by inverting 5-6 times. Pipette or pour the entire solution onto the S.N.A.P.™ Miniprep Column/Collection Tube.
8. Centrifuge the S.N.A.P.™ Miniprep Column/Collection Tube at room temperature at 1,000-3,000 x g for 30 seconds.
   Alternatively, let the **Binding Buffer** flow through the column by gravity for 10-15 minutes. The plasmid DNA is now bound to the column.
9. Discard the column flow through.
10. Add 500 µl of **Wash Buffer.**
11. Centrifuge the S.N.A.P.™ Miniprep Column/Collection Tube at room temperature at 1,000-3,000 x g for 10-30 seconds or allow to drain by gravity for 7 minutes. Discard the column flow through.
12. Add 900 µl of **1X Final Wash** and centrifuge or drain as in Step 11. (The 1X Final Wash was made in **Before Starting**, Step 2.)
13. Centrifuge the S.N.A.P.™ Miniprep Column/Collection Tube at room temperature at maximum speed for 1 minute to dry the resin.

Plasmid Elution

14. To elute the plasmid DNA, transfer the S.N.A.P.™ Miniprep Column to a sterile microcentrifuge tube and add 60 µl of TE buffer or sterile water directly to the resin. Incubate for 3 minutes at room temperature.
15. Centrifuge the S.N.A.P.™ Miniprep Column/Collection Tube at room temperature at maximum speed for 30 seconds. The plasmid DNA is now eluted from the column. Remove and discard the column..

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Determination of DNA Yield

The plasmid DNA is now pure and ready for all molecular biology applications. To determine concentration of plasmid DNA, see below. To determine plasmid DNA yield, you can use a spectrophotometer or Invitrogen's DNA DipStick™ Kit (Cat. no. K5632-01). When using a spectrophotometer, blank first with water or TE at 260 nm. Use 5-10 µl of the plasmid DNA sample to determine concentration. The formula below should be used:

$$[DNA] = (A_{260}) (0.05 \text{ mg/ml}) \times D$$

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

Troubleshooting

Use cell cultures that are 1 to 1.5 x 10^9 cells/ml, OD_{600} equals 1.5 to 2.0 per ml. We recommend LB medium. Do not use TB (Terrific Broth) because the cell culture will become too dense and will clog the S.N.A.P.™ Miniprep Column.

Low yield may be caused by one or more of the following reasons:

Reason	Solution
Incomplete lysis (Step 3)	Decrease amount of cell culture to 1-1.5 x 10^9 cells/ml, OD_{600} = 1.5 to 2.0 /ml. Incomplete lysis is caused by using too many cells.
Incomplete precipitation (Step 4)	Decrease amount of cell culture to 1-1.5 x 10^9 cells/ml, OD_{600} = 1.5 to 2.0 /ml. Incomplete precipitation is caused by using too many cells.
Low copy number plasmid	Increase amount of culture to 4-6 ml and process as 3 samples. Follow miniprep procedure to Step 7 for each sample. Then add all three samples to the same column before proceeding to Step 10. This will bind all of the plasmid DNA in the samples to the column before washing and eluting.
Centrifugation speed too high	Do not centrifuge higher than 5,000 rpm in Steps 6-13. Plasmid yields will decrease 10%.
Elution in <60 µl	Always elute DNA in 60 µl. Elution in <60 µl may result in a decrease in plasmid yield.
Genomic DNA contamination	Perform Steps 3-5 with gentle mixing to avoid damaging genomic DNA. Genomic DNA must be intact to be efficiently precipitated away from the plasmid DNA.