S.N.A.P.™ Gel Purification Kit

For Rapid Purification of DNA Fragments from Agarose Gels

Catalog nos. K1999-25

Version C
082701
25-0351

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Important Information

Shipping/Storage

The S.N.A.P.™ Gel Purification Kit is shipped and stored at room temperature.

Kit Contents

The components of the S.N.A.P.™ Gel Purification Kit are described in the table below.

Component	Composition	Amount
Purification Columns	--	25
Collection Vials	--	25
Sodium Iodide Solution (NaI solution)	6.6 M Sodium Iodide 16 mM Sodium Sulfite	11 ml
Binding Buffer	7 M Guanidinium HCl	15 ml
4X Final Wash	400 mM NaCl	6 ml

S.N.A.P.™ Columns

Each lot of S.N.A.P.™ columns is qualified as follows:

Ten columns from each lot are randomly selected and tested as described below.

Binding Capacity: 20 µg of pre-purified control plasmid is bound to the column and eluted. Yield of eluted DNA is determined by the OD_{260} and should be > 10 µg.

Visual Inspection: Five samples of plasmid DNA isolated from a bacterial culture using the S.N.A.P.™ protocol were visualized on a 0.8% agarose gel. Only supercoiled plasmid should be present with no contaminating RNA.

Restriction Digest: Five 500 ng plasmid DNA samples are each digested with 4 units of *Apa* I for 90 minutes. When analyzed by agarose gel electrophoresis, the digest should be 99% complete.

Endonuclease Activity: Five 500 ng plasmid DNA samples are incubated with 10 mM Mg^{2+} for 4 hours and analyzed on a 0.8% agarose gel. No degradation should be observed.

S.N.A.P.™ Gel Purification Kit

To functionally test the S.N.A.P.™ Gel Purification Kit, a PCR product was purified from a 0.8% agarose gel using the protocol on pages 2-3. The purified PCR product was analyzed on a second 0.8% agarose gel to confirm recovery. No degradation should be observed.

Gel-Purifying DNA

Introduction

The procedure below is appropriate for isolating a DNA fragment or a PCR product in a volume of approximately 100 µl. We've purified fragments or PCR products as small as 100 bp and as large as 6 kb.

Materials Supplied by the User

You will need the following reagents and equipment for gel purification

- Apparatus for agarose mini-gel electrophoresis with 8-lane or 12-lane comb
- General purpose agarose
- 1X TAE buffer (50 mM Tris-acetate, pH 8, 1 mM EDTA. **Note: Do not use TBE gels. Borate interferes with sodium iodide**)
- Clean glass flask
- Autoclaved water or TE buffer
- Sterile 25-30 ml bottle to prepare 1X Final Wash (see below)
- 100% ethanol
- New razor blade
- 42°C to 50°C water bath
- Microcentrifuge
- Sterile microcentrifuge tubes

Before Starting

Bring the Sodium Iodide solution, Binding Buffer, and 4X Final Wash to room temperature. Mix well before using. Please note that these solutions may be stored at room temperature.

To prepare 1X Final Wash, transfer all of the 4X Final Wash solution (6 ml) to a sterile 25-30 ml bottle. Add 18 ml of 100% **ethanol** to the 4X Final Wash solution to prepare the 1X Final Wash solution (24 ml). Store at room temperature.

Nuclease Control

It is very important to minimize the presence of nucleases to ensure purification of high quality DNA. Please follow the guidelines listed below. While some guidelines may not appear as rigorous as others they are sufficient for purifying DNA.

- Wear gloves at all times
- Use sterile plasticware and glassware
- Autoclave TAE to use as the running buffer
- Rinse agarose gel apparatus and comb with autoclaved water or TE buffer
- Use a new razor to excise gel slice*
- Use new plastic wrap (i.e. Saran® Wrap) if needed

*The same razor may be used to excise different bands in the same gel if you are careful not to bring over pieces from an earlier excision.

Continued on next page

Gel-Purifying DNA, Continued

Preparing the Gel

Follow the instructions below to prepare a 1% agarose gel. The recipe will make one agarose gel with a volume of 50 ml.

1. Mix 0.5 g general purpose agarose and 50 ml 1X TAE buffer in a clean glass flask.
2. Place flask in the microwave and heat until just boiling. Swirl gently to mix and dissolve the agarose and continue to heat in this fashion for 3 minutes to destroy nucleases.

   Caution: Vigorous swirling can cause the superheated agarose to boil out of the flask.
3. Remove from the microwave and cool for 3 minutes.
4. Rinse the gel box and comb with autoclaved water or TE buffer. **Note**: Use a comb that will hold 1-5 µg DNA in one well. Wells should be as small as possible to minimize the volume of the gel slice.
5. Pour the gel and set the comb in the gel.
6. When the gel has solidified, cover the gel with 1X TAE buffer and gently remove the comb.
7. Load your sample (1-5 µg DNA) and run the gel. Do not run the DNA into the gel too far, as the band will become too diffuse for excision. You want to keep the gel slice as small as possible (**100 µl total volume**).
8. Stain in a 0.5-1 µg/µl ethidium bromide solution.

Excising DNA

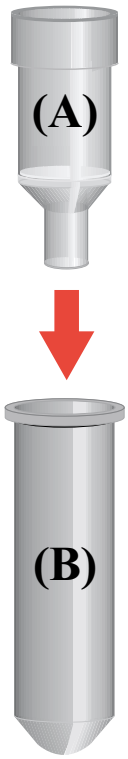
1. Pour off the stain (or transfer the gel to new Saran® Wrap). Visualize gel under UV light.
2. Using a new razor blade, carefully excise the DNA from the gel. **Note**: Razor blade may be rinsed with autoclaved water or TE prior to cutting the next band.
3. Transfer the excised plug of agarose to a sterile 1.5 ml microcentrifuge tube.

   Note: You may cut the agarose plug into small pieces to reduce the melting time and temperature.
4. Estimate the volume of the agarose (generally this is around 100 µl). Alternatively, you can weigh the gel slice and assume that 1 mg ~ 1 µl.
5. Add 2.5 times its volume of 6.6 M sodium iodide (i.e. 250 µl) and mix by shaking vigorously by hand or vortexing.
6. Incubate at 42 to 50°C until the agarose is **completely** melted (~5 minutes). Mix the solution periodically by vortexing.
7. Place the tube at room temperature and add 1.5 volumes of Binding Buffer (i.e. 525 µl) and mix well. Proceed directly to **Isolation of DNA,** next page.

Continued on next page

Gel-Purifying DNA, Continued

Isolating DNA



1. Assemble a S.N.A.P.™ purification column (A) and collection vial (B) and load all of the mixture from Step 7, above, onto the column (875 µl).
2. Centrifuge at 2,000 to 3,000 x g in a microcentrifuge for 30 seconds at room temperature.
3. Pour the liquid in the collection vial **back onto the column** and repeat Step 2.
4. Repeat Step 3 one more time to bind all the DNA to the column (i.e. load solution onto the column for a total of 3 times).
5. After the last centrifugation, discard the liquid in the collection tube.
6. Add 400 µl of 1X Final Wash to the S.N.A.P.™ column and centrifuge as in Step 2.
7. Repeat Step 6 and discard the liquid in the collection tube after the second centrifugation (800 µl).
8. Centrifuge the column again **at maximum speed (>10,000 x g) for at least 1 minute** to dry the column resin. Discard the collection vial.
9. Transfer the column to a new, sterile 1.5 ml microcentrifuge tube.
10. Add 40 µl of sterile water directly to the column material and incubate for 1 minute at room temperature to let the buffer absorb into the column.
11. Centrifuge the column at maximum speed (>10,000 x g) for 1 minute to elute the DNA into the microcentrifuge tube.
12. Place the tube on ice and discard the column.
13. Assay 10 µl by ethidium bromide agarose gel electrophoresis to estimate the DNA concentration. Concentration should be between 2 and 40 ng/µl. In most cases, there is no need to concentrate the DNA further.

Store the DNA at -20°C or proceed directly to the next application.

Technical Service

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http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact us

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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

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3E Company
Voice: 1-760-602-8700

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