AxyPrep DNA Gel Extraction Kit

For the rapid purification of DNA fragments from agarose gels

Kit contents, storage and stability

Cat. No.	AP-GX-4	AP-GX-50	AP-GX-250
Kit size	4 preps	50 preps	250 preps
Miniprep column	4	50	250
2 ml microfuge tube	4	50	250
1.5 ml microfuge tube	4	50	250
Buffer DE-A	6 ml	66 ml	2x165 ml
Buffer DE-B	3 ml	33 ml	165 ml
Buffer W1	2.8 ml	28 ml	135 ml
Buffer W2 concentrate	2.4 ml	24 ml	2x72 ml
Eluent	1 ml	5 ml	25 ml
Protocol manual	1	1	1

All buffers in this kit are stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight or extremes in temperature.

Buffer DE-A: Gel solubilization buffer. Store at room temperature.
Buffer DE-B: Binding buffer. Store at room temperature.
Buffer W1: Wash buffer. Store at room temperature.
Buffer W2 concentrate: Desalting buffer. Before use, add the amount of ethanol specified. Store at room temperature. Either 100% or 95% denatured ethanol can be used.
Eluent: 2.5 mM Tris-Cl, pH 8.5. Store at room temperature.

Introduction

The AxyPrep DNA Gel Extraction Kit employs optimized reagents in combination with a convenient Miniprep column to purify DNA fragments from either TAE or TBE agarose gels (regular and low-melt). Each Miniprep column will bind up to 8 μg of DNA. DNA fragments in a size range of 70 bp to 10 kb can be efficiently recovered. Depending upon the length of the DNA fragments, the recovery rate is approximately 60-85%. Buffer DE-A contains a reagent for solubilizing agarose gels, in combination with a second reagent that protects DNA fragments against degradation during heating. DNA fragments purified by this method are full-length with high biological activity. These fragments are suitable for all routine molecular biology applications, such as ligation, PCR, sequencing, etc.

Caution

Buffers DE-A, DE-B and W1 contain chemical irritants. When working with the buffers, always wear suitable protective clothing such as safety glasses, laboratory coat and gloves. Be careful to avoid contact with eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek medical assistance.

Equipment and consumables required

- Heated water bath or temperature block
- AxyVac Vacuum manifold with luer-type fittings (#AP-VM)
- Vacuum source and regulator (-25-30 inches Hg required)
- Microcentrifuge capable of 12,000xg
- 100% or 95% (denatured) ethanol
- 100% isopropanol

Preparation before experiment

1) Before using the kit, add amount of ethanol specified on the bottle label to the Buffer W2 concentrate. Either 100% or 95% (denatured) ethanol can be used. Mix well and store at room temperature.
2) Adjust water bath or temperature block to 75°C.
3) Pre-warming Eluent to 65°C will generally improve elution efficiency.

Protocols:

DNA Gel Extraction Vacuum Protocol

Any vacuum manifold with complementary fittings, such as the AxyVac Vacuum Manifold can be used with the Miniprep columns. A negative pressure of -25-30 inches Hg is be required. We recommend the use of a vacuum regulator to adjust the negative pressure.

Note: -25-30 inches Hg is equivalent to -850-1,000 mbar and -12-15 psi.

1. Excise the agarose gel slice containing the DNA fragment of interest with a clean, sharp scalpel under ultraviolet illumination. Briefly place the excised gel slice on absorbent toweling to remove residual buffer. Transfer the gel slice to a piece or plastic wrap or a weighing boat. Mince the gel into small pieces and weigh. In this application, the weight of gel is regarded as equivalent to the volume. For example, 100 mg of gel is equivalent to a 100 μl volume. Transfer the gel slice into a 1.5 ml microfuge tube.

   Note: Alternatively, the gel slice can be placed into the 1.5 ml microfuge tube and then crushed with a pipette tip or other suitable device. Spin the tube for 30 sec at 12,000xg to consolidate the gel at the bottom of the tube. Use the graduations to estimate the volume of the agarose gel.

2. Add a 3x sample volume of Buffer DE-A.

   Note: The color of Buffer DE-A is red. This color is used to add contrast in the next step, so that any pieces of unsolubilized agarose can be visualized.

3. Resuspend the gel in Buffer DE-A by vortexing. Heat at 75°C until the gel is completely dissolved (typically, 6-8 minutes). Heat at 40°C if low-melt agarose gel is used. Intermittently vortexing (every 2-3 minutes) will accelerate gel solubilization.

   IMPORTANT: Gel must be completely dissolved or the DNA fragment recovery will be reduced.

   IMPORTANT: Do not heat the gel for longer than 10 minutes.

4. Add 0.5x Buffer DE-A volume of Buffer DE-B, mix. If the DNA fragment is less than 400 bp, supplement further with a 1x sample volume of isopropanol.

   Example: For a 1% gel slice equivalent to 100 μl, add the following:

   - 300 μl Buffer DE-A
   - 150 μl Buffer DE-B

   If the DNA fragment is <400 bp, you would also add:

   - 100 μl of isopropanol.

   Note: The color of the mixture will turn yellow after the addition of Buffer DE-B. Please make sure the contents are a uniform yellow color before proceeding.

5. Attach the vacuum manifold to a vacuum source. Position a Miniprep column securely into one of the complementary fittings. Transfer the binding mix from Step 4 to the Miniprep column(s). Switch on the vacuum source and adjust the negative pressure to -25-30 inches Hg. Continue to apply vacuum until no liquid remains in the Miniprep column.
6. Pipette 500 μl of Buffer W1 into the Miniprep column(s). Draw all liquid through the column(s).
7. Pipette 700 μl of Buffer W2 along the wall of the Miniprep column(s) to wash off all residual Buffer W1. Draw all liquid through the column(s).

   Note: Make sure that ethanol has been added into Buffer W2 concentrate.

   Note: Be sure to add Buffer W2 along the tube wall to wash off all residual salt.

8. Repeat this wash step with a second 700 μl aliquot of Buffer W2.

   Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions, such as ligation and sequencing reaction.

9. Transfer the Miniprep column into a 2 ml microfuge tube (provided) and centrifuge at 12,000xg for 1 minute to purge residual Buffer W2 from the binding membrane.
10. Transfer the Miniprep column into a clean 1.5 ml microfuge tube (provided). To elute the DNA, add 25-30 μl of Eluent or deionized water to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000xg for 1 minute.

    Note: Pre-warming the Eluent to 65°C will generally improve elution efficiency.

    Note: Deionized water can also be used to elute the DNA fragments.

DNA Gel Extraction Spin Protocol

1. Excise the agarose gel slice containing the DNA fragment of interest with a clean, sharp scalpel under ultraviolet illumination. Briefly place the excised gel slice on absorbent toweling to remove residual buffer. Transfer the gel slice to a piece or plastic wrap or a weighing boat. Mince the gel into small pieces and weigh. In this application, the weight of gel is regarded as equivalent to the volume. For example, 100 mg of gel is equivalent to a 100 µl volume. Transfer the gel slice into a 1.5 ml microfuge tube.

   Note: Alternatively, the gel slice can be placed into the 1.5 ml microfuge tube and then crushed with a pipette tip or other suitable device. Spin the tube for 30 sec at 12,000xg to consolidate the gel at the bottom of the tube. Use the graduations to estimate the volume of the agarose gel.

2. Add a 3x sample volume of Buffer DE-A.

   Note: The color of Buffer DE-A is red. This color is used to add contrast in the next step, so that any pieces of unsolubilized agarose can be visualized.

3. Resuspend the gel in Buffer DE-A by vortexing. Heat at 75°C until the gel is completely dissolved (typically, 6-8 minutes). Heat at 40°C if low-melt agarose gel is used. Intermittent vortexing (every 2-3 minutes) will accelerate gel solubilization.

   IMPORTANT: Gel must be completely dissolved or the DNA fragment recovery will be reduced.

   IMPORTANT: Do not heat the gel for longer than 10 minutes.

4. Add 0.5x Buffer DE-A volume of Buffer DE-B, mix. If the DNA fragment is less than 400 bp, supplement further with a 1x sample volume of isopropanol.

   Example: For a 1% gel slice equivalent to 100 µl, add the following:

   - 300 µl Buffer DE-A
   - 150 µl Buffer DE-B

   If the DNA fragment is <400 bp, you would also add:

   - 100 µl of isopropanol.

   Note: The color of the mixture will turn yellow after the addition of Buffer DE-B. Please make sure the contents are a uniform yellow color before proceeding.

5. Place a Miniprep column into a 2 ml microfuge tube (provided). Transfer the solubilized agarose from Step 4 into the column. Centrifuge at 12,000xg for 1 minute.
6. Discard the filtrate from the 2 ml microfuge tube. Return the Miniprep column to the 2 ml microfuge tube and add 500 µl of Buffer W1. Centrifuge at 12,000xg for 30 seconds.
7. Discard the filtrate from the 2 ml microfuge tube. Return the Miniprep column to the 2 ml microfuge tube and add 700 µl of Buffer W2. Centrifuge at 12,000xg for 30 seconds.

   Note: Make sure that 95-100% ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

8. Discard the filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Add a second 700 µl aliquot of Buffer W2 and centrifuge at 12,000xg for 1 minute.

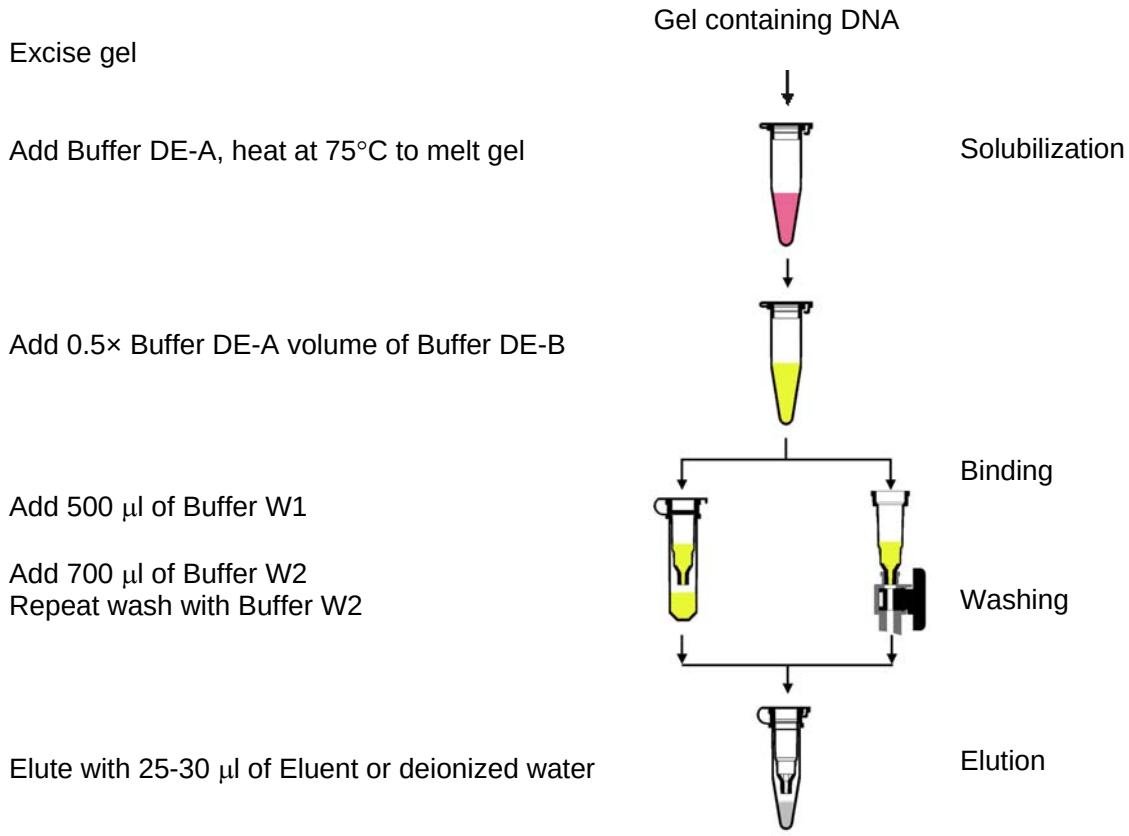
   Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions, such as ligation and sequencing reaction.

9. Discard the filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Centrifuge at 12,000xg for 1 minute.
10. Transfer the Miniprep column into a clean 1.5 ml microfuge tube (provided). To elute the DNA, add 25-30 μl of Eluent or deionized water to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000xg for 1 minute.

Note: Pre-warming the Eluent at 65°C will generally improve elution efficiency.

Note: Deionized water can also be used to elute the DNA fragments.

Overview



Troubleshooting

1. Low or no recovery

Gel not completely solubilized

Incomplete solubilization of the agarose gel will allow the DNA fragments to be masked from the AxyPrep membrane surface, preventing interaction and binding. Depending upon the amount of incompletely solubilized gel remaining in the sample, only partial binding of the fragments may occur, resulting in premature elution and fragments loss during the ensuing wash steps. Usually, this is attributable to processing too much agarose (too large and/or too high percentage). Be sure to use the correct amount of Buffer DE-A. Carefully inspect the sample during heating to be sure that no solid agarose remains. Use frequent vortexing during heating to enhance solubilization.

Poor fragment binding

To ensure complete solubilization of the agarose, increase the amount of Buffer DE-A to 4× the sample volume. Trim the gel as close to the DNA fragments as possible to minimize the amount of agarose processed. Be sure to supplement the solubilized agarose containing DNA fragments <400 bp with 1× sample volume of isopropanol (100%).

Premature elution of bound DNA fragments

As described above, premature elution of the DNA fragments can be attributable to the presence of excessive agarose. In addition, omission of the ethanol from the Buffer W2 or misformulation with 70% ethanol (instead of 95-100%) will also cause the DNA fragments to detach during the desalting step.

Poor elution efficiency

Do not allow the Miniprep column to remain under vacuum for an excessive period of time after the last Buffer W2 wash step. To improve elution efficiency, heat the eluent to 65°C.

2. DNA fragments do not perform well in enzymatic reactions

Residual salt

Be sure to perform 2× Buffer W2 washes.

Residual ethanol

Spin the column for 1 additional minute (2 minutes total) after the last Buffer W2 wash step.

Residual agarose

To ensure complete removal of the agarose be sure that the agarose slice is fully solubilized by Buffer DE-A. Try to trim the gel as close to the DNA fragments as possible to minimize the amount of agarose processed.

For technical inquiries about AxyPrep Kits, please contact Axygen Biosciences at *support.axyprepkits@axygenbio.com*