

## AxyPrep DNA Gel Extraction Kit

### Kit contents, storage and stability

Cat. No.	AP-GX-50	AP-GX-250
Kit size	50 preps	250 preps
AxyPrep Columns	50	250
2 ml microfuge tube	50	250
1.5 ml microfuge tube	50	250
Buffer DE-A	2 × 33 ml	2 × 165 ml
Buffer DE-B	33 ml	165 ml
Buffer W1	28 ml	135 ml
Buffer W2 concentrate	24 ml	2 × 72 ml
Eluent	5 ml	25 ml
Protocol Manual	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 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 AxyPrep spin column to purify DNA fragments from either TAE or TBE agarose gels (regular and low-melt). Each AxyPrep column will bind up to 8 µg of DNA. DNA fragments in a size range of 70 bp up to 10 kb can be efficiently recovered. Depending upon the length of the DNA fragment, 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, in vitro transcription, PCR, sequencing, microinjection, 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 temp block
- Vacuum manifold with luer-type fittings (#AP-VAC)
- Vacuum source and regulator (-25-30 inches Hg required)
- Microcentrifuge capable of  $12,000 \times g$
- 100% or 95% (denatured) ethanol
- 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 temp block to 75°C.
- 3) Pre-warming the Eluent to 65°C will generally improve elution efficiency.

## Protocols:

### DNA Gel Extraction Vacuum Protocol

Any vacuum manifold with complimentary fittings, such as the AxyPrep Vacuum Manifold can be used with the AxyPrep 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 of 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  $\mu$ l 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  $12,000 \times g$  to consolidate the gel at the bottom of the tube. Use the graduations to estimate the volume of the agarose gel.

2. Add a 3 × sample volume of Buffer DE-A.
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 0.5 × Buffer DE-A volume of Buffer DE-B, mix. If the DNA fragment is less than 400 bp, supplement further with a 1 × 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.

5. Attach the vacuum manifold to a vacuum source. Position an AxyPrep column securely into one of the complimentary fittings. Transfer the binding mix from Step 4 to the AxyPrep 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 AxyPrep column.
6. Pipette 500 µl of Buffer W1 into the AxyPrep column(s). Draw all liquid through the column.
7. Pipette 700 µl of Buffer W2 along the wall of the AxyPrep column to wash off all residual Buffer W1. Draw all liquid through the column.
8. Repeat this wash step with a second 700 µl aliquot of Buffer W2.
9. Transfer the AxyPrep column into a 2 ml microfuge tube (provided) and centrifuge at 12,000 × g for 1 minute to purge residual W2 from the binding membrane.
10. Transfer the AxyPrep column to a clean 1.5 ml microfuge tube (provided). To elute the DNA, add 25-30 µl of Eluent to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000 × g 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.

## 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 of 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  $12,000 \times g$  to consolidate the gel at the bottom of the tube. Use the graduations to estimate the volume of the agarose gel.

2. Add a  $3 \times$  sample volume of Buffer DE-A.
3. Resuspend the gel in Buffer DE-A by vortexing. Heat at  $75^{\circ}\text{C}$  until the gel is completely dissolved (typically, 6-8 minutes). Heat at  $40^{\circ}\text{C}$  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  $0.5 \times$  Buffer DE-A volume of Buffer DE-B, mix. If the DNA fragment is less than 400 bp, supplement further with a  $1 \times$  sample volume of isopropanol.

**Example:** For a 1% gel slice equivalent to  $100 \mu\text{l}$ , add the following:

- $300 \mu\text{l}$  Buffer DE-A
- $150 \mu\text{l}$  Buffer DE-B

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

- $100 \mu\text{l}$  of isopropanol.

5. Place an AxyPrep column into a 2 ml microfuge tube. Transfer the solubilized agarose from Step 4 into the column. Centrifuge at  $12,000 \times g$  for 1 minute.
6. Discard the filtrate from the 2 ml microfuge tube. Return the AxyPrep column to the 2 ml microfuge tube and add  $500 \mu\text{l}$  of Buffer W1. Centrifuge at  $12,000 \times g$  for 30 sec.
7. Discard the filtrate from the 2 ml microfuge tube. Return the AxyPrep column to the 2 ml microfuge tube and add  $700 \mu\text{l}$  of Buffer W2. Centrifuge at  $12,000 \times g$  for 30 sec.

**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. **Optional Step:** Discard the filtrate from the 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Add  $700 \mu\text{l}$  of Buffer W2 to the AxyPrep column and centrifuge at  $12,000 \times g$  for 1 minute.

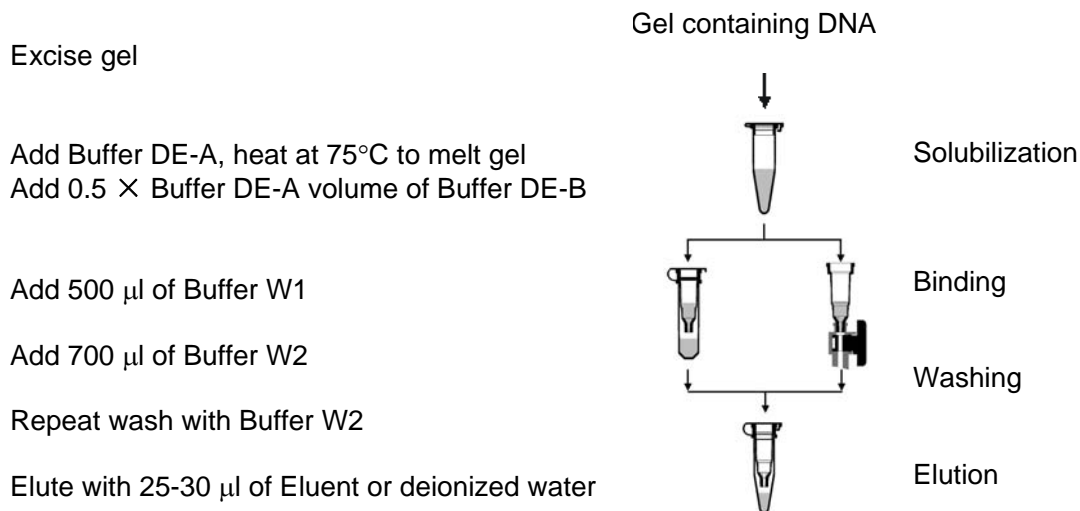
**Note:** Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions, such as ligation and sequencing reaction.

9. Discard filtrate from 2 ml microfuge tube. Place the AxyPrep column back into the 2 ml microfuge tube. Centrifuge at  $12,000 \times g$  for 1 minute.
10. Transfer the AxyPrep column to a clean 1.5 ml microfuge tube (provided). To elute the DNA, add  $25\text{-}30 \mu\text{l}$  of Eluent to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at  $12,000 \times g$  for 1 minute.

**Note:** Pre-warming the Eluent at  $65^{\circ}\text{C}$  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 fragment loss during the ensuing wash steps. Usually, this is attributable to processing too much agarose (too large and/or too high a 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 fragment as possible to minimize the amount of agarose processed. Be sure to supplement the solubilized agarose containing DNA fragments <400 bp with 1/5th 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 AxyPrep column to remain under vacuum for an excessive period of time after the last Buffer W2 wash. To improve elution efficiency, heat the eluent to 65°C.

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## 2. DNA fragments do not perform well in enzymatic reactions

### Residual salt

### Residual ethanol

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

### 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 fragment as possible to minimize the amount of agarose processed.