Zymoclean™ Gel DNA Recovery Kit

Zymo Research

Abstract

Highlights

- Quick (15 minute) high-yield recovery of ultra-pure DNA from agarose gels.
- Column design permits DNA elution at high concentrations into minimal volumes (≥ 6 µl).
- Eluted DNA is well suited for use in DNA ligation, sequencing, labeling, PCR, etc.

Citation: Zymo Research Zymoclean™ Gel DNA Recovery Kit. **protocols.io** dx.doi.org/10.17504/protocols.io.gitbuen
Published: 21 Dec 2016

Guidelines

Product Contents

Zymoclean™ Gel DNA Recovery Kit (Kit Size)	D4001, D4007 (50 Preps.)	D4002, D4008 (200 Preps.)	Storage Temperature
ADB	50 ml	2x100 ml	Room Temp.
DNA Wash Buffer[1]	6 ml	24 ml	Room Temp.
DNA Elution Buffer	1 ml	4 ml	Room Temp
Zymo-Spin™ I Columns	50 D4001 – uncapped columns D4007 – capped columns	200 D4002 – uncapped columns D4008 – capped columns	Room Temp.
Collection Tubes	50	200	Room Temp.
Instruction Manual	1	1	-

Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

[1] Ethanol must be added prior to use as indicated on the **DNA Wash Buffer** label.

Specifications

- **DNA Purity** – High-quality, purified DNA is especially well suited for sequencing and ligation reactions.

- **DNA Size Limits** – From ~50 bp to 23 kb.

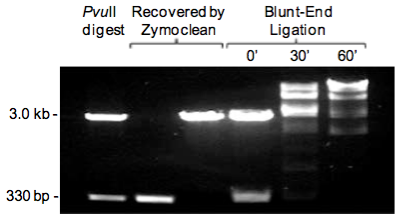
- **DNA Recovery** – Typically, up to 5 µg total DNA per column can be eluted into as little as 6 µl of low salt **DNA Elution Buffer** or water. For DNA 50 bp to 10 kb, the recovery is 70-90%. For DNA 11 kb to 23 kb, the recovery is 50-70%.

- **Sample Sources** – DNA in excised agarose gel slices.

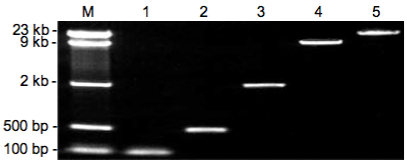
- **Product Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS.

Product Description

The **Zymoclean™ Gel DNA Recovery Kit** provides a hassle-free method for high yield recovery of pure DNA from agarose gels. Simply add the specially formulated **Agarose Dissolving Buffer (ADB)** to the gel slice containing your DNA sample, let dissolve, and then transfer to the supplied **Zymo-Spin™ Column**. There is no need for organic denaturants or chloroform. Instead, the product utilizes Fast-Spin column technology to yield high-quality DNA in just 15 minutes (See figures below). DNA purified using the **Zymoclean™ Gel DNA Recovery Kit** is perfectly suited for use in DNA ligation reactions, sequencing, DNA labeling reactions, PCR, etc.



Blunt-end ligation of DNA fragments purified using the Zymoclean™ Gel DNA Recovery Kit. Fragments from plasmid DNA digested with *Pvu* II restriction endonuclease were purified, then mixed and ligated for the indicated times.



Effectiveness of the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: DNA from ladder that was excised and recovered from gel.

Zymoclean™ products are offered in single column (uncapped or capped column) or 96-well format. In addition, the **Zymoclean™ Large Fragment DNA Recovery Kit** is designed for large DNA (up to

200 kb) gel recovery.

Available Formats

	Uncapped Column	Capped Column	96- well	Capped Column
				
			High-throughput	For Large DNA
Capacity	5 µg/ prep.	5 µg/ prep.	5 µg/ well.	10 µg/ prep.
Elution Vol.	≥ 6 µl	≥ 6 µl	≥ 10 µl	≥ 10 µl
Cat. Nos.	D4001, D4002	D4007, D4008	D4021, D4022	D4045, D4046

Buffer Preparation

Before starting: Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **DNA Wash Buffer** concentrate. Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate.

Troubleshooting

Low Recovery

- **Ensure Agarose is Fully Dissolved**
  There may be small globules of undissolved agarose in the sample that can interfere with DNA recovery by clogging the column and leeching salts into the eluate.

- **Gel Dissolved at Temperatures Above 60 °C**
  If dissolved at a higher temperature, DNA may be denatured affecting recovery. For optimal results, dissolve the gel slice between 37-55 °C.

- **Improperly Prepared/Stored DNA Wash Buffer**
  Make sure ethanol has been added to the DNA Wash Buffer concentrate. Cap the bottle tightly to prevent evaporation over time.

- **Addition of DNA Elution Buffer**

Add elution buffer directly to the column matrix, not to the walls of the column. Elution buffer requires contact with the matrix for at least 1 minute for large DNA ≥ 10kb.

- **Incomplete Elution**

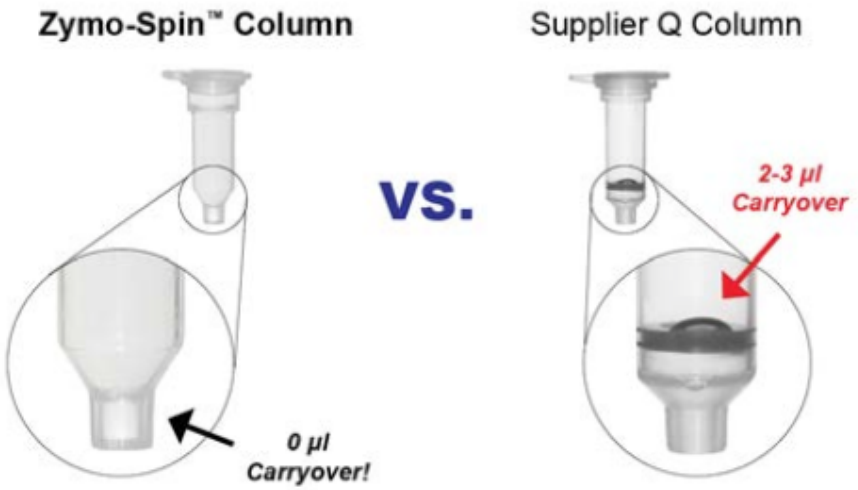
1. DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥ 50 kb), apply heated elution buffer (60-70 °C) to the column and incubate for several minutes prior to elution.

2. Sequential elutions may be performed for quantitatively higher recovery but lower final DNA concentration. This is recommended for DNA ≥ 10 kb.

Low A260/A230 ratio

- **Column tip contaminated**

When removing the column from the collection tube, be careful that the tip of the column does not come into contact with the flowthrough. Trace amounts of salt from the flowthrough can contaminate a sample resulting in a low A_{260}/A_{230} ratio. Ethanol contamination from the flowthrough can also interfere with DNA elution. Zymo-Spin™ columns are designed for complete elution with no buffer retention or carryover (see below).



Following Clean-up with the DCCTM, Multiple Bands Appear in an Agarose Gel

- **Acidification of DNA Loading Dye**

Most loading dyes do not contain EDTA and will acidify (pH ≤ 4) over time due to some microbial growth. This low pH is enough to cause DNA degradation. Therefore, if water is used to elute the DNA, 6X Loading Dye containing 1 mM EDTA is recommended.

Before start

Before starting: Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **DNA Wash Buffer** concentrate. Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate.

Materials

Zymoclean™ Gel DNA Recovery Kit D4001 by Zymo Research

Protocol

Step

Step 1.
Excise the DNA fragment[1] from the agarose gel using a razor blade, scalpel or other device and transfer it into a 1.5 ml microcentrifuge tube.

NOTES
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[1]The amount of agarose excised from the gel should be as small as possible.

Step 2.
Add 3 volumes of **ADB** to each volume of agarose excised from the gel (e.g. for 100 µl (mg) of agarose gel slice add 300 µl of **ADB**).

Step 3.
Incubate at 37-55 °C for 5-10 minutes until the gel slice is completely dissolved[2].

DURATION
00:10:00

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For DNA fragments > 8 kb, following the incubation step, add one additional volume (equal to that of the gel slice) of water to the mixture for better DNA recovery (*e.g.,* 100 µl agarose, 300 µl **ADB**, and 100 µl water).

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[2] Do not incubate above 60°C. It is important that the gel slice dissolve completely. This can be facilitated by gentle mixing during the incubation.

Step 4.

Transfer the melted agarose solution to a **Zymo-Spin™ Column** in a **Collection Tube.**

Step 5.
Centrifuge for 30-60 seconds. Discard the flow-through[3].

DURATION
00:01:00

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[3] Remove the flow-through by aspiration. Avoid contamination of the collection tube rim.

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All centrifugation steps should be performed between 10,000 - 16,000 x g.

Step 6.
Add 200 µl of **DNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through. (wash 1/2)

DURATION
00:00:30

Step 7.

Add 200 µl of **DNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through. (wash 2/2)

DURATION
00:00:30

Step 8.
Add ≥ 6 µl **DNA Elution Buffer**[4] or water[5] directly to the column matrix.

NOTES
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[4] **DNA Elution Buffer:** 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA.

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[5] Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH

is >6.0. Waiting 1 minute prior to elution may improve the yield of larger (> 6 kb) DNA. For even larger DNA (> 10 kb), the total yield may be improved by eluting the DNA with 60-70 ºC **DNA Elution Buffer.**

Step 9.

Place column into a 1.5 ml tube and centrifuge for 30-60 seconds to elute DNA.

DURATION
00:01:00

NOTES
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All centrifugation steps should be performed between 10,000 - 16,000 x g.

Step 10.
Ultra-pure DNA is now ready for use.

Warnings

All centrifugation steps should be performed between 10,000 - 16,000 x g.