Zymoclean™ Gel DNA Recovery Kit

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Abstract

This is a protocol for high yield recovery of pure DNA from agarose gels using the Zymoclean™ Gel DNA Recovery Kit.

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Guidelines

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

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

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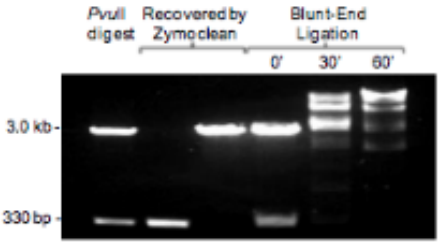
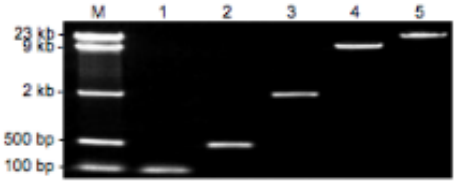
The **Zymoclean™ Gel DNA Recovery Kit** provides a hassle-free method for high yield recovery of pure DNA from agarose gels. Simply add the specially formulated **Agarose Dissolving Buffer (ADB)** to the gel slice containing your DNA sample, let dissolve, and then transfer to the supplied **Zymo-Spin™ 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

Before start

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

✓ ADB (Agarose Dissolving Buffer) D4001-1-50 by Contributed by users

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Zymo DNA Wash Buffer D4003-2-6 by Zymo Research

Zymo DNA Elution Buffer D3004-4-1 by Zymo Research

Zymoclean™ Gel DNA Recovery Kit - Uncapped columns D4001 by Zymo Research

Protocol

Step 1.
Excise the DNA fragment from the agarose gel using a razor blade, scalpel or other device and transfer it into a 15 ml microcentrifuge tube

NOTES
Alan Cone 13 Jul 2015
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.

REAGENTS
ADB (Agarose Dissolving Buffer) D4001-1-50 by Contributed by users

NOTES
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(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.

DURATION
00:10:00

NOTES
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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.
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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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I like to let the agarose dissolve for 15 minutes at 55 °C versus the Zymo recommendation.

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.

DURATION
00:01:00

NOTES
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Remove the flow-through by aspiration. Avoid contamination of the collection tube rim.

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Step 6.
Wash #1:Add 200 µl of DNA Wash Buffer to the column.

AMOUNT
200 µl Additional info:

REAGENTS
Zymo DNA Wash Buffer D4003-2-6 by Zymo Research

Step 7.
Wash #1:Centrifuge for 30 seconds. Discard the flow-through.

DURATION
00:00:30

Step 8.
Wash #2: Add 200 µl of DNA Wash Buffer to the column.

AMOUNT
200 µl Additional info:

REAGENTS
Zymo DNA Wash Buffer D4003-2-6 by Zymo Research

NOTES
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Ultra-pure DNA is now ready for use.

Step 9.
Wash #2:Centrifuge for 30 seconds. Discard the flow-through.

DURATION
00:00:30

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

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 o C DNA Elution Buffer

Step 10.
Add ≥ 6 µl DNA Elution Buffer or water directly to the column matrix.

AMOUNT
6 µl Additional info:

REAGENTS
Zymo DNA Elution Buffer D3004-4-1 by Zymo Research

NOTES
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I use exactly 6 uL of their elution buffer.

Step 11.
Place column into a 1.5 ml tube and centrifuge for 30-60 seconds to elute DNA.

DURATION
00:01:00

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