

ZR-96 Zymoclean™ Gel DNA Recovery Kit

High-quality DNA from TAE/TBE-buffered agarose gels.

Highlights

- Quick, high-throughput (96-well plate) recovery of pure DNA from agarose gels.
- Unique Zymo-Spin™ plate facilitates binding of up to 5 µg DNA/well and elution with ≥ 15 µl/well.
- Eluted DNA is well suited for use in DNA ligation, sequencing, labeling, PCR, *etc.*

Catalog Numbers:
D4021, D4022



Scan with your smart-phone camera to
view the online protocol/video.



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Product Contents

ZR-96 Zymoclean™ Gel DNA Recovery Kit	D4021 (2 x 96 Preps.)	D4022 (4 x 96 Preps.)	Storage Temperature
ADB	100 ml	2 x 100 ml	Room Temp.
DNA Wash Buffer ¹	24 ml	48 ml	Room Temp.
DNA Elution Buffer	10 ml	16 ml	Room Temp.
Zymo-Spin™ I-96 Plate	2 Plates	4 Plates	Room Temp.
Collection Plate	2 Plates	4 Plates	Room Temp.
Elution Plate	2 Plates	4 Plates	Room Temp.
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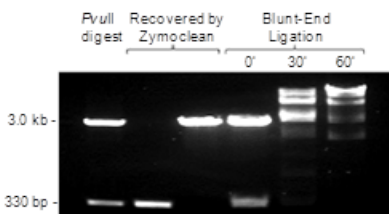
¹ Ethanol must be added prior to use as indicated on the **DNA Wash Buffer** label.

Specifications

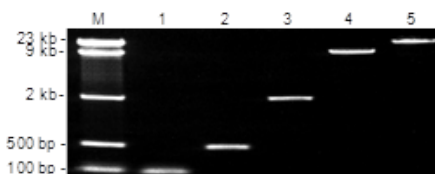
- **DNA Purity** – High-quality, purified DNA is especially well suited for sequencing and ligation reactions.
- **DNA Size Limits** – From ~50 bp to 23 kb.
- **DNA Recovery** – Typically, up to 5 µg total DNA per well can be eluted with ≥ 15 µl low salt **DNA Elution Buffer** or water. For DNA 75 bp to 10 kb the recovery is 70- 90%. For DNA 11 kb to 23 kb the recovery is 50-70%.
- **Sample Sources** – DNA excised from agarose gels.
- **Product Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS.
- **Equipment** – microcentrifuge, vortex, centrifuge with microplate carriers.

Product Description

Zymo Research's **ZR-96 Zymoclean™ Gel DNA Recovery Kit** provides a hassle-free method for high yield high-throughput (96-well plate) recovery of pure DNA from agarose gels. Simply add the specially formulated **Agarose Dissolving Buffer (ADB)** to the gel slices containing DNA, let dissolve, and then transfer to the wells of the supplied **Zymo-Spin™ I-96 Plate**. There is no need for organic denaturants or chloroform. Instead, the product utilizes *Fast-Spin* technology to yield high-quality DNA in just minutes. DNA purified using the **ZR-96 Zymoclean™ Gel DNA Recovery Kit** is well suited for DNA ligation, sequencing, DNA labeling, PCR, *etc.*







Blunt-ended 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 amount of time.



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

Formats

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.

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

Protocol

Buffer Preparation

- ✓ Before starting: Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate. Add 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **DNA Wash Buffer** concentrate.

Sample Processing

All centrifugation steps should be performed between 3,000 - 5,000 x g.

1. Excise the DNA fragment from the agarose gel using a razor blade, scalpel or other device and transfer it into a well of the provided **Collection Plate**.

Note: The amount of agarose excised from the gel should be as small as possible and should not exceed 150 µl (150 mg) per well.

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**).
3. Incubate at 37-55°C for 5-10 minutes until the gel slice is completely dissolved¹.

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).

4. Transfer the melted agarose solutions to the wells of the **Zymo-Spin™ I-96 Plate** on the empty **Collection Plate** used in Step 1 (above).
5. Centrifuge for 5 minutes until the sample mixtures have been completely filtered. Discard the flow-through in the **Collection Plate**².
6. Add 300 µl of **DNA Wash Buffer** to each well of the **Zymo-Spin™ I-96 Plate**. Centrifuge for 5 minutes. Repeat the wash step, but centrifuge for 15 minutes.

¹ Do not incubate above 60°C. It is important that the gel slice dissolves completely. This can be facilitated by gentle mixing during the incubation.

² Remove the flow-through by aspiration. Avoid contamination of the rims of the wells.

7. Add ≥ 15 μ l **DNA Elution Buffer**³ or water⁴ directly to the column matrix in each well. Transfer the **Zymo-Spin™ I-96 Plate** onto an **Elution Plate** and centrifuge for 3 minutes to elute the DNA.

Ultra-pure DNA is now ready for use.

³ **DNA Elution Buffer:** 10mM Tris-HCl, pH 8.5, 0.1mM EDTA.

⁴ 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°C **DNA Elution Buffer**.

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 ≥ 10 kb.

- **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 A_{260}/A_{230} 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.

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

- **Acidification of DNA Loading Dye**

Most loading dyes do not contain EDTA and will acidify ($\text{pH} \leq 4$) 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.

Ordering Information

Product Description	Catalog No.	Size
Zymoclean™ Gel DNA Recovery Kit <i>Supplied with uncapped columns</i>	D4001T D4001 D4002	10 Preps. 50 Preps. 200 Preps.
Zymoclean™ Gel DNA Recovery Kit <i>Supplied with capped columns</i>	D4007 D4008	50 Preps. 200 Preps.
Zymoclean™ Large Fragment Gel DNA Recovery Kit <i>Supplied with capped columns</i>	D4045 D4046	25 Preps. 100 Preps.
ZR-96 Zymoclean™ Gel DNA Recovery Kit <i>Supplied with 96-well plates</i>	D4021 D4022	2 x 96 Preps. 4 x 96 Preps.

Individual Kit Components	Catalog No.	Amount
ADB	D4001-1-50 D4001-1-100	50 ml 100 ml
DNA Wash Buffer (concentrate)	D4003-2-6 D4003-2-24	6 ml 24 ml
DNA Elution Buffer	D3004-4-10 D3004-4-16	10 ml 16 ml
Zymo-Spin™ I-96 Plate	C2004	2 Plates
Collection Plate	C2002	2 Plates
Elution Plate	C2003	2 Plates



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