ZYMO RESEARCH

DNA Purification Made Simple

Zymoclean™ Gel DNA Recovery Kit

For rapid purification of high-quality DNA from TAE/TBE-buffered agarose gels.

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.*

Catalog Numbers:
D4001T, D4001, D4002, D4007, D4008

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

Zymoclean™ Gel DNA Recovery Kit	D4001T (10 Preps.)	D4001, D4007 (50 Preps.)	D4002, D4008 (200 Preps.)	Storage Temperature
ADB (Agarose Dissolving Buffer)	10 ml	50 ml	2 x 100 ml	Room Temp.
DNA Wash Buffer[1]	6 ml	6 ml	24 ml	Room Temp.
DNA Elution Buffer	1 ml	1 ml	4 ml	Room Temp.
Zymo-Spin™ I Columns	10 uncapped	50 D4001 – uncapped D4007 – capped	200 D4002 – uncapped D4008 – capped	Room Temp.
Collection Tubes	10	50	200	Room Temp.
Instruction Manual	1	1	1	-

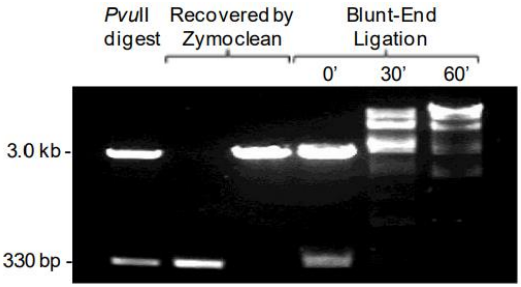
[1] Ethanol must be added prior to use as indicated on the **DNA Wash Buffer** label. DNA Wash Buffer included with D4001S and D4001T is supplied ready-to-use and does not require the addition of ethanol prior to use.

Specifications

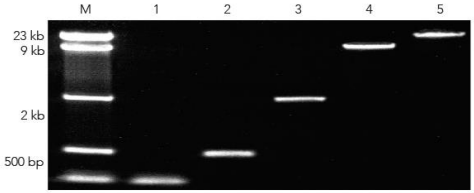
- **DNA Purity –** High-quality, purified DNA is especially well suited for sequencing and ligation reactions.
- **DNA Size Limits –** From ~50 bp to 23 kb.
- **DNA Recovery –** Typically, up to 5 µg total DNA per column can be eluted into as little as 6 µl of low salt **DNA Elution Buffer** or water. 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.

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
				
			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

Protocol

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.

- ✓ **DNA Wash Buffer** included with D4001S and D4001T is supplied ready-to-use and does not require the addition of ethanol prior to use.

Sample Processing

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

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.

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 55 °C[2] for a minimum of 10 minutes[3] and then briefly mix the sample by vortexing or inverting. For optimal performance, it is essential that the gel slice is completely dissolved before moving on to step 4.

   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 solution to a **Zymo-Spin™ Column** in a **Collection Tube**.

5. Centrifuge for 1 minute. Discard the flow-through[4].

6. Add 200 µl of **DNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through. Repeat the wash step.

---

[1] The amount of agarose excised from the gel should be as small as possible.
[2] Do not incubate above 60 °C.
[3] The incubation time will vary depending on the percentage of agarose and mass of the gel slice. This step can be performed at 37 °C but will require longer incubation to completely dissolve the agarose.
[4] Remove the flow-through by aspiration. Avoid contamination of the collection tube rim.

7. Add ≥ 6 µl **DNA Elution Buffer**[4] or water[5] directly to the column matrix. Place column into a 1.5 ml tube and centrifuge for 1 minute to elute DNA.

   Ultra-pure DNA is now ready for use.

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

Troubleshooting

Problem	Possible Causes and Suggested Solutions
Low Recovery	**Ensure Agarose is Fully Dissolved.** There may be small globules of undissolved agarose in the sample that can reduce DNA recovery by clogging the column and interfering with DNA Binding and elution. **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 Buffe**r 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.** 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. 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. **Ensure Agarose is Fully Dissolved.** There may be small globules of undissolved agarose in the sample that can reduce DNA quality by clogging the column and leaching salts into the eluate.

Problem	Possible Causes and Suggested Solutions
Following Clean-up with ZymoClean™, 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.

Ordering Information

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

Refer to Page 1 for column design specifics in each kit

Individual Kit Components	Catalog No.	Amount
ADB (Agarose Dissolving Buffer)	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	D3004-4-1 D3004-4-4 D3004-4-10	1 ml 4 ml 10 ml
Zymo-Spin™ I Columns (uncapped)	C1003-50 C1003-250	50 Pack 250 Pack
Zymo-Spin™ IC Columns (capped)	C1004-50 C1004-250	50 Pack 250 Pack
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 Pack 500 Pack 1000 Pack

Complete Your Cloning Workflow

✓ **Transfection-grade plasmid DNA from a miniprep**

ZymoPURE™ Plasmid Miniprep	Size	Catalog No.
ZymoPURE™ Plasmid Miniprep Kit	10 Preps. 50 Preps. 100 Preps. 400 Preps. 800 Preps.	D4208T D4309 D4210 D4211 D4212

✓ **20 Minute Endotoxin-Free Midi & Maxipreps**

ZymoPURE™ II Plasmid Prep Kits	Size	Catalog No.
ZymoPURE™ II Plasmid Midiprep Kit	25 Preps. 50 Preps.	D4200 D4201
ZymoPURE™ II Plasmid Maxiprep Kit	10 Preps. 20 Preps.	D4202 D4203
ZymoPURE™ II Plasmid Gigaprep Kit	5 Preps.	D4204

✓ **Simple 20 second High Efficiency Transformations**

Mix & Go! Competent Cells	Size	Catalog No.
DH5α	10 x 100 µl aliquots 96 x 50 µl aliquots 96 x 50 µl aliquots PCR Plate	T3007 T3009 T3010
JM109	10 x 100 µl aliquots 96 x 50 µl aliquots	T3019 T3020
Zymo10B	10 x 100 µl aliquots 96 x 50 µl aliquots	T3003 T3005
HB101	10 x 100 µl aliquots 96 x 50 µl aliquots	T3011 T3013
TG1	10 x 100 µl aliquots	T3017

✓ **Recover ultra-pure highly concentrated DNA from PCR & other sources**

DNA Clean & Concentrator™	Size	Catalog No.
DNA Clean & Concentrator™-5	50 Preps. 200 Preps.	D4003 D4004
ZR-96 DNA Clean-Up Kit™	2 x 96 Preps. 4 x 96 Preps.	D4017 D4018

✓ **Rapid extraction of total RNA from any sample**

Quick-RNA Kits ™	Size	Catalog No.
***Quick*-RNA Miniprep Plus Kit**	50 Preps. 200 Preps.	R1057 D1058
***Quick*-RNA Microprep Kit**	50 preps. 200 Preps.	R1050 R1051

Notes

Notes

100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

ZYMO RESEARCH

The **BEAUTY** *of* **SCIENCE** *is to Make Things* **SIMPLE**®

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682