

Zymoclean™ Large Fragment DNA Recovery Kit

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

Highlights

- Quick (15 minute) spin column recovery of large-sized DNA (e.g., genomic, plasmid (BAC/PAC), viral, phage, etc.) from agarose gels. *No messy precipitations!*
- Unique column design for low volume (≥ 10 μ l) elution of ultra-pure, high-yield DNA.
- Eluted DNA is well suited for use in PCR, sequencing, endonuclease digestion, ligation, etc.

Catalog Numbers:
D4045, D4046



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



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Product Contents

Zymoclean™ Large Fragment DNA Recovery Kit	D4045 (25 Preps.)	D4046 (100 Preps.)	Storage Temperature
ADB (Agarose Dissolving Buffer)	50 ml	100 ml	Room Temp.
DNA Wash Buffer ¹	6 ml	24 ml	Room Temp.
DNA Elution Buffer	1 ml	4 ml	Room Temp.
Zymo-Spin™ IC-XL Columns	25	100	Room Temp.
Collection Tubes	50	200	Room Temp.
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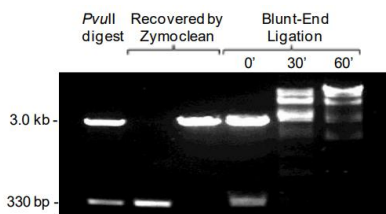
¹ Ethanol must be added prior to use as indicated on the **DNA Wash Buffer** label.

Specifications

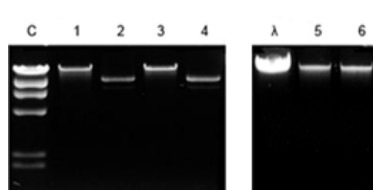
- **DNA Purity** – High-quality, purified DNA is especially well suited for sequencing and ligation reactions.
- **DNA Size Limits** – From ~50 bp to >200 kb.
- **DNA Recovery** – Typically, up to 10 µg total DNA per column can be eluted into ≥10 µl of low salt **DNA Elution Buffer** or water. Recovery of DNA ranges from 70-95%
- **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™ Large Fragment DNA Recovery Kit** provides a streamlined method for the rapid purification and concentration of high-quality large-sized DNA from agarose gels. Simply add the specially formulated **Agarose Dissolving Buffer (ADB)** to the gel slice containing a DNA sample, let dissolve, and then transfer to the supplied **Zymo-Spin™ IC-XL Column**. There is no need for organic denaturants or chloroform. Instead, the product utilizes unique spin column technology to yield high-quality, purified DNA in just minutes. DNA purified using the **Zymoclean™ Large Fragment DNA Recovery Kit** is ideal for PCR, sequencing, endonuclease digestion, ligation, *etc.* The entire procedure typically takes about 15 minutes.







Blunt-ended ligation of DNA fragments purified using the Zymoclean™ Large Fragment DNA Recovery Kit. Fragments from plasmid DNA digested with PvuII were purified, then mixed and ligated for the times indicated in the figure (above).



DNA recovery (lanes 1-6) from HindIII-digested lambda DNA (lane C) and lambda DNA (λ) using the Zymoclean™ Large Fragment DNA Recovery Kit. Lanes 1, 3: 23 kb and 2, 4: 9 kb bands, respectively. Lanes 5, 6: ~48 kb bands.

Formats

Zymoclean™ products are offered in single column (uncapped or capped column) or 96-well format. The **Zymoclean™ Gel DNA Recovery Kit** is ideal for purifying DNA from 50 bp – 10 kb with recovery into $\geq 6 \mu\text{l}$.

	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.	$\geq 6 \mu\text{l}$	$\geq 6 \mu\text{l}$	$\geq 10 \mu\text{l}$	$\geq 10 \mu\text{l}$
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.

Sample Processing

All centrifugation steps should be performed between 11,000 - 16,000 x g.

1. Excise the DNA fragment¹ from the agarose gel using a razor blade or scalpel 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 °C² for a minimum of 10 minutes³ 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.
4. Transfer the melted agarose solution to a **Zymo-Spin™ Column** in a **Collection Tube**.
5. Centrifuge for 1 minute. Discard the flow-through⁴.
6. Add 200 µl of **DNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through. Repeat the wash step.
7. Add ≥ 10 µl **DNA Elution Buffer**⁵ or water⁶ directly to the column matrix and wait for 1 minute. Place column into a 1.5 ml tube and centrifuge for 30 seconds to elute DNA.

Ultra-pure DNA is now ready for use.

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.

⁵ **DNA Elution Buffer:** 10mM Tris-HCl, pH 8.5, 0.1mM EDTA.

⁶ Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. 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	<p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p>
Low A_{260}/A_{230} ratio	<p>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.</p> <p>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.</p>

Problem	Possible Causes and Suggested Solutions
<p>Following Clean-up with ZymoClean™, Multiple Bands Appear in an Agarose Gel</p>	<p>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.</p>

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 (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™ IC-XL Columns (capped)	C1002-25 C1002-50	25 Pack 50 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

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