



**ZYMO RESEARCH**

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

## Zymoclean™ Large Fragment DNA Recovery Kit

Catalog Nos. **D4045 & D4046**

### 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 ( $\geq 10 \mu\text{l}$ ) elution of ultra-pure, high-yield DNA.
- Eluted DNA is well suited for use in PCR, sequencing, endonuclease digestion, ligation, etc.

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

## Product Contents

<b>Zymoclean™ Large Fragment DNA Recovery Kit (Kit Size)</b>	<b>D4045 (25 Preps.)</b>	<b>D4046 (100 Preps.)</b>	<b>Storage Temperature</b>
<b>ADB</b>	50 ml	100 ml	Room Temp.
<b>DNA Wash Buffer<sup>1</sup></b>	6 ml	24 ml	Room Temp.
<b>DNA Elution Buffer</b>	1 ml	4 ml	Room Temp
<b>Zymo-Spin™ IC-XL Columns</b>	25	100	Room Temp.
<b>Collection Tubes</b>	50	200	Room Temp.
<b>Instruction Manual</b>	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.

<sup>1</sup> 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.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. 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.

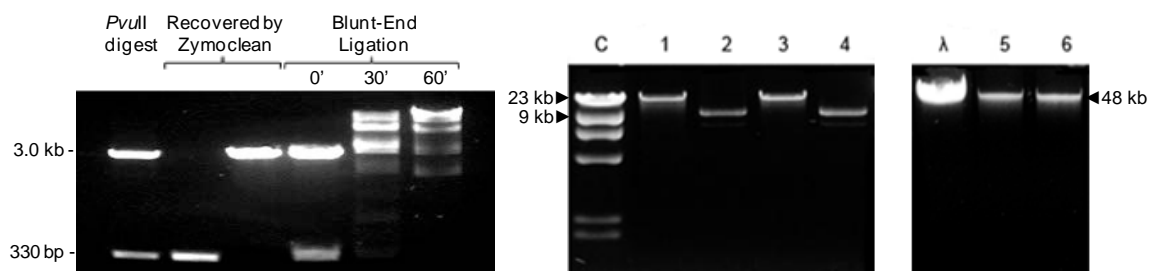
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## Product Description

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.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail [tech@zymoresearch.com](mailto:tech@zymoresearch.com).







**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.

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

### Available Formats

	Uncapped Column	Capped Column	96- well	Capped Column
				
			High-throughput	For Large DNA
Capacity	5 $\mu\text{g}$ / prep.	5 $\mu\text{g}$ / prep.	5 $\mu\text{g}$ / well.	10 $\mu\text{g}$ / 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

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## **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.

## **Protocol**<sup>1</sup>

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

1. Excise the DNA fragment<sup>1</sup> from the agarose gel using a razor blade or scalpel and transfer it to 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 37-55 °C for 5-10 minutes until the gel slice is completely dissolved<sup>2</sup>.
4. Transfer the melted agarose solution to a **Zymo-Spin™ Column** in a **Collection Tube**.
5. Centrifuge for 1 minute. Discard the flow-through<sup>3</sup>.
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**<sup>4</sup> or water<sup>5</sup> 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.

### **Notes:**

<sup>1</sup> The amount of agarose excised from the gel should be as small as possible.

<sup>2</sup> 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.

<sup>3</sup> Remove the flow-through by aspiration. Avoid contamination of the collection tube rim.

<sup>4</sup> **DNA Elution Buffer:** 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA.

<sup>5</sup> 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**

### ***Low Recovery***

- **Ensure Agarose is Fully Dissolved**

There may be small globules of undissolved agarose in the sample, containing DNA inaccessible for recovery. Undissolved agarose can also inhibit 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  $\geq 10$ kb.

- **Incomplete Elution**

1. DNA elution is dependent on pH, temperature, and time. For large genomic DNA ( $\geq 50$  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  $\geq 10$  kb.

### ***Low A<sub>260</sub>/A<sub>230</sub> 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.	Kit Size (Preps.)
<b>Zymoclean™ Gel DNA Recovery Kit</b> <i>Supplied with uncapped columns</i>	D4001	50
	D4002	200
<b>Zymoclean™ Gel DNA Recovery Kit</b> <i>Supplied with capped columns</i>	D4007	50
	D4008	200
<b>Zymoclean™ Large Fragment Gel DNA Recovery Kit</b> <i>Supplied with capped columns</i>	D4045	25
	D4046	100
<b>ZR-96 Zymoclean™ Gel DNA Recovery Kit</b> <i>Supplied with 96-well plates</i>	D4021	2 x 96
	D4022	4 x 96

*Refer to Page 2 for column design specifics in each kit.*

For Individual Sale	Catalog No.	Size
<b>ADB</b>	D4001-1-50	50 ml
	D4001-1-100	100 ml
<b>DNA Wash Buffer (concentrate)</b>	D4003-2-6	6 ml
	D4003-2-24	24 ml
<b>DNA Elution Buffer</b>	D3004-4-1	1 ml
	D3004-4-4	4ml
	D3004-4-10	10 ml
<b>Collection Tubes</b>	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1000	1000 tubes

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## Popular Products From Zymo Research

Product	Description	Kit Size (Preps.)	Catalog No. (Format)
<b>DNA Clean-up, Concentration &amp; Recovery</b>			
DNA Clean & Concentrator™-5	Clean and concentrate up to 5 µg DNA into ≥6 µl elution volume in as little as 2 minutes with no wash residue carryover.	50 200 50 200	D4003 (uncapped) D4004 (uncapped) D4013 (capped) D4014 (capped)
DNA Clean & Concentrator™-25	Clean and concentrate 25 µg of DNA into ≥25 µl elution volume in as little as 2 minutes with no wash residue carryover.	50 200 50 200	D4005 (uncapped) D4006 (uncapped) D4033 (capped) D4034 (capped)
ZR-96 DNA Clean & Concentrator™-5	Quick (30 minute), high throughput recovery of up to 5 µg pure DNA into 10-15 µl minimum elution volume allows for highly concentrated DNA.	2 x 96 4 x 96	D4023 D4024
Genomic DNA Clean & Concentrator™	Quick (5 minute) clean-up of up to 10 µg high molecular weight DNA (≥ 20kb - 200 kb) from any enzymatic reaction or impure preparation without precipitations.	25 100	D4010 D4011
Zymoclean™ Gel DNA Recovery Kit	Purify DNA from high and low-melting agarose gels in minutes.	50 200 50 200	D4001 (uncapped) D4002 (uncapped) D4007 (capped) D4008 (capped)
ZR-96 Zymoclean™ Gel DNA Recovery Kit	High-throughput DNA purification from high and low-melting agarose gels.	2 x 96 4 x 96	D4021 D4022
Zymoclean™ Large Fragment DNA Recovery Kit	Purify high molecular weight DNA (≥ 20 kb - 200 kb) from high and low-melting agarose gels in minutes.	25 100	D4045 D4046
OneStep™ PCR Inhibitor Removal Kit	Fast, one step procedure for removal of PCR inhibitors such as polyphenolics, humic/fulvic acids, melanin, etc. for successful PCR and other downstream applications.	50 2 x 96	D6030 D6035
<b>Plasmid DNA Purification</b>			
Zyppy™ Plasmid Miniprep Kit	Pellet-Free™ plasmid DNA purification in less than 10 minutes. Recover up to 25 µg DNA in as low as 30 µl.	50 100 400	D4036 D4019 D4020
Zyppy™-96 Plasmid Miniprep	The fastest and simplest high-throughput method for plasmid purification. Magnetic bead format available for automated liquid handling platforms.	2 x 96 4 x 96 8 x 96 2 x 96 4 x 96 8 x 96	D4041 (spin plate) D4042 (spin plate) D4043 (spin plate) D4100 (magnetic bead) D4101 (magnetic bead) D4102 (magnetic bead)
Zyppy™ Plasmid Midiprep Kit	Pellet-Free™ plasmid DNA purification in 15 minutes in a 150 µl minimum elution volume.	25 50	D4025 D4026
ZR Plasmid MiniPrep™-Classic	Plasmid DNA purification in minutes: (alkaline lysis/spin column format for low 30 µl elution volume).	100 400 800	D4015 D4016 D4054
<b>Genomic DNA Purification</b>			
Quick-gDNA™ MiniPrep	Easy purification from whole blood, plasma, serum, body fluids, buffy coat, tissue, swabs or cultured cells ≥15 minutes <u>without</u> the use of Proteinase K or organic denaturants.	50/200 50/200	D3006/D3007 uncapped) D3024/D3025 (capped)
ZR Genomic DNA™-Tissue MiniPrep	High quality DNA purification from <u>solid tissues</u> (e.g., tail snips, ear punches, adipose tissue, etc.), body fluids, cultured cells, buccal cells, FFPE tissues, hair, and other biological sources using Proteinase K and Fast.	50/200 50/200	D3050 D3051
Environmental DNA Purification Kits	Unique BashingBead™ technology allows isolation of DNA from samples refractory to conventional lysis procedures including tough-to-lyse tissues, soil samples, feces, plants, seeds, insects, bacteria, yeast, filamentous fungi, unicellular and filamentous algae, and protozoa	Spin Column & 96-well Plate	Visit <a href="#">website for a comprehensive list</a>
<b>RNA Purification</b>			
RNA Clean & Concentrator™-5	Clean and concentrate up to 5 µg RNA into ≥6 µl elution volume in as little as 5 minutes with no wash residue carryover.	50 200	R1015 R1016
Direct-Zol™ RNA MiniPrep	Quick, spin column purification of high-quality (DNA-free) total RNA <b>directly</b> from TRI-Reagent® or similar acid-guanidinium-phenol based reagents (TRIzol®, RNAzol®, QIAzol®, TriPure, RNA-Bee etc.).	50 200	R2051 R2053
ZR RNA MiniPrep	Quick (15 minute) RNA isolation from a variety of sources using <i>Fast-Spin</i> column technology without the use of organic denaturants..	50 200	R1064 R1065

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## Epigenetics Products From Zymo Research

Product	Description	Kit Size	Cat. No. (Format)
<b>Bisulfite Kits for DNA Methylation Detection</b>			
<b>EZ DNA Methylation™ Kit</b>	For the conversion of unmethylated cytosines in DNA to uracil via the <u>chemical-denaturation</u> of DNA and a specially designed CT Conversion Reagent. <i>Fast-Spin</i> technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis. Magnetic bead format for automated liquid handling platforms.	50/200 Rxns. 2 x 96 Rxns. 2 x 96 Rxns. 4 x 96 Rxns.	<b>D5001/D5002</b> (column) <b>D5003</b> (shallow-well plate) <b>D5004</b> (deep-well plate) <b>D5040</b> (magnetic bead)
<b>EZ DNA Methylation-Gold™ Kit</b>	For the fast (3 hr.) conversion of unmethylated cytosines in DNA to uracil via <u>heat/chemical-denaturation</u> of DNA and a specially designed CT Conversion Reagent. <i>Fast-Spin</i> technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis. Magnetic bead format for automated liquid handling platforms.	50/200 Rxns. 2 x 96 Rxns. 2 x 96 Rxns. 4 x 96 Rxns.	<b>D5005/D5006</b> (column) <b>D5007</b> (shallow-well plate) <b>D5008</b> (deep-well plate) <b>D5042</b> (magnetic bead)
<b>EZ DNA Methylation-Direct™ Kit</b>	Simple and reliable DNA bisulfite conversion directly from blood, tissue (FFPE/LCM), and cells without the prerequisite for DNA purification in as little as 4-6 hrs. The increased sensitivity of this kit makes it possible to amplify bisulfite converted DNA from as few as 10 cells or 50 pg DNA. Magnetic bead format for adaptation to automated liquid handling platforms.	50/200 Rxns. 2 x 96 Rxns. 2 x 96 Rxns. 4 x 96 Rxns.	<b>D5020/D5021</b> (column) <b>D5022</b> (shallow-well plate) <b>D5023</b> (deep-well plate) <b>D5044</b> (magnetic bead)
<b>EZ DNA Methylation-Lightning™ Kit</b>	Complete bisulfite conversion in about an hour using a unique liquid format conversion reagent. <i>Fast-Spin</i> technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis. Magnetic bead format for automated liquid handling platforms.	50/200 Rxns. 2 x 96 Rxns. 2 x 96 Rxns. 4 x 96 Rxns.	<b>D5030/D5031</b> (column) <b>D5032</b> (shallow-well plate) <b>D5033</b> (deep-well plate) <b>D5046</b> (magnetic bead)
<b>EZ DNA Methylation-Startup™ Kit</b>	Designed for the first time user requiring a consolidated product to perform DNA methylation analysis. Includes technologies for sample processing, bisulfite treatment of DNA, and PCR amplification of "converted" DNA for methylation analysis.	1 Kit	<b>D5024</b>
<b>Methylated DNA Standards</b>			
<b>Universal Methylated Human DNA Standard</b>	Human (male) genomic DNA having all CpG sites methylated. To be used for the evaluation of bisulfite-mediated conversion of DNA. Supplied with a control primer set.	1 Set	<b>D5011</b>
<b>Universal Methylated Mouse DNA Standard</b>	Mouse (male) DNA having all CpG sites methylated. To be used for the evaluation of bisulfite-mediated conversion of DNA. Supplied with a control primer set.	1 Set	<b>D5012</b>
<b>Region-Specific DNA Methylation Screening</b>			
<b>OneStep qMethyl™ Kit</b>	Single step real-time PCR procedure for bisulfite-free determination of DNA methylation status. Available without fluorescent dye for probe-based detection (Lite).	1 x 96 Rxns. 1 x 96 Rxns.	<b>D5310</b> <b>D5311</b> (Lite)
<b>OneStep qMethyl™ Array</b>	Premade 96-well assay for bisulfite-free determination of region-specific DNA methylation assessment in the promoter region of any one of the following prominent tumor suppressor genes: RASSF1, RARB, CDKN2A (p16), MGMT, or CCND2.	1 x 96 Rxns.	<b>D5312</b>
<b>Epigenetics Services</b>			
For more information, visit <a href="http://www.zymoresearch.com/services">http://www.zymoresearch.com/services</a> or inquire at <a href="mailto:services@zymoresearch.com">services@zymoresearch.com</a> .			
<b>Services for Methylated DNA Analysis</b>			
Simplify biomarker discovery with our 5-mC Analysis platforms that combine Zymo's well-established bisulfite technologies with next-generation sequencing for the most comprehensive DNA methylation analysis services available.			
<b>Services for Hydroxymethylated DNA Analysis</b>			
Novel genome-wide 5-hmC analysis platform featuring cutting-edge 5-hmC DNA enrichment, library prep, and next-generation sequencing technologies to ensure the sensitivity of 5-hmC detection in genome-wide context.			
<b>Hydroxymethylation Detection</b>			
<b>Quest 5-hmC™ DNA Enrichment Kit</b>	Featuring J-base binding protein (JBP) for the specific enrichment of 5-hmC containing DNA, the consolidated workflow makes the procedure reliable for robust analysis of multiple samples.	25 Rxns. 50 Rxns.	<b>D5420</b> <b>D5421</b>
<b>Quest 5-hmC™ DNA ELISA Kit</b>	Streamlined workflow for both the direct and relative quantitation of 5-hmC, in a global genomic context, with a robust colorimetric readout.	1 x 96 Rxns. 2 x 96 Rxns.	<b>D5425</b> <b>D5426</b>
<b>Anti-5-Hydroxymethylcytosine Polyclonal Antibody</b>	Polyclonal antibody has been engineered to maximize sensitivity to low amounts of hydroxymethylated gDNA while minimizing crossreactivity with unmodified or methylated cytosine residues. The antibody is suitable for use in ELISA, IP, and immunohistochemical labeling.	50 µg 200 µg	<b>A4001-50</b> <b>A4001-200</b>
<b>DNA Degradase™ DNA Degradase Plus™</b>	Whole genomic DNA can be treated with these enzyme cocktails for processing to individual nucleotides (Degradase™) or nucleosides (Degradase Plus™) for interrogation in chromatographic and spectroscopic methods including TLC, LC/MS, MALDI-TOF, and more.	500 U 2000 U 250 U 1000 U	<b>E2016</b> <b>E2017</b> <b>E2020</b> <b>E2021</b>
<b>Other...</b>			
<b>Zymo Taq™ DNA Polymerase</b>	Zymo Taq™ "hot start" DNA Polymerase is specifically designed for the amplification of "difficult" DNA templates including: bisulfite-treated DNA for methylation detection. The product generates specific amplicons with little or no by-product formation.	50 Rxns. 200 Rxns	<b>E2001/E2001</b> (system) <b>E2003/E2004</b> (premix)
<b>Methylated-DNA IP Kit</b>	IP with a highly specific anti-5-methylcytosine monoclonal antibody. Designed for the enrichment of 5-methylcytosine-containing DNA from any pool of fragmented genomic DNA for use in genome-wide methylation analysis.	10 Rxns.	<b>D5101</b>

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