ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR-96 Zymoclean™ Gel DNA Recovery Kit

Catalog Nos. **D4021 & D4022**

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.

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ZYMO RESEARCH CORP.

Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

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

Product Contents

ZR-96 Zymoclean™ Gel DNA Recovery Kit (Kit Size)	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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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.

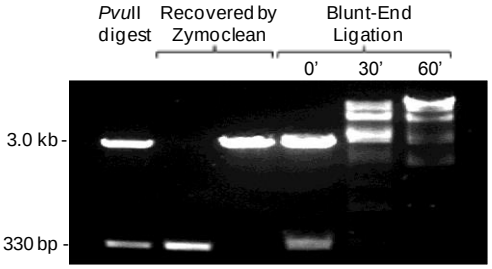
[1] Ethanol must be added prior to use as indicated on **DNA Wash Buffer** label.

Specifications

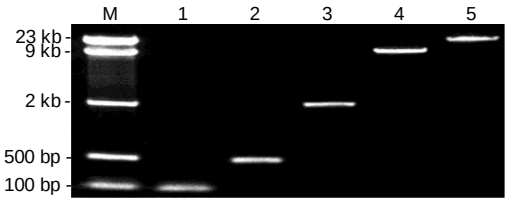
- **DNA Purity** – High-quality, purified DNA is especially well suited for sequencing and ligation reactions.
- **DNA Size Limits** – From ~50 bp to 23 kb.
- **DNA Recovery** – Typically, up to 5 µg total DNA per well can be eluted with ≥ 15 µl low salt **DNA Elution** Buffer or water. For DNA 75 bp 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) Buffer** 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.

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 200kb) 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

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

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.

Protocol

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 dissolved[1].

   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.

7. Add ≥15 µl **DNA Elution Buffer**[3] or water[4] 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.

Notes:

[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.

[3] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM 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 ≥ 10kb.
- **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 A260/A230 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 (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.	Kit Size (Preps.)
Zymoclean™ Gel DNA Recovery Kit *Supplied with **uncapped columns***	D4001 D4002	50 200
Zymoclean™ Gel DNA Recovery Kit *Supplied with **capped columns***	D4007 D4008	50 200
Zymoclean™ Large Fragment Gel DNA Recovery Kit *Supplied with **capped columns***	D4045 D4046	25 100
ZR-96 Zymoclean™ Gel DNA Recovery Kit *Supplied with 96-well plates*	D4021 D4022	2 x 96 4 x 96

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

For Individual Sale	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	D3004-4-16 D3004-4-10	16 ml 10 ml
Zymo-Spin™ I-96 Plate	C2004	2 plates
Collection Plate	C2002	2 plates
Elution Plate	C2003	2 plates

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Popular Products From Zymo Research

Product	Description	Kit Size (Preps.)	Catalog No. (Format)
DNA Clean-up, Concentration & Recovery			
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**
Plasmid DNA Purification			
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**
Genomic DNA Purification			
***Quick-gDNA*™ MiniPrep**	Easy purification from whole blood, plasma, serum, body fluids, buffy coat, tissue, swabs or cultured cells ≥15 minutes without the use of Proteinase K or organic denaturants.	50/200 50/200	**D3006/D3007** uncapped) **D3024/D3025** (capped)
ZR Genomic DNA™-Tissue MiniPrep	igh quality DNA purification from solid tissues (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 website for a comprehensive list**
RNA Purification			
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 ***directly*** 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 *Fast-Spin* column technology without the use of organic denaturants..	50 200	**R1064** **R1065**

Epigenetics Products From Zymo Research

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Product	Description	Kit Size	Cat. No. (Format)
Bisulfite Kits for DNA Methylation Detection			
EZ DNA Methylation™ Kit	For the conversion of unmethylated cytosines in DNA to uracil via the chemical-denaturation of DNA and a specially designed CT Conversion Reagent. *Fast-Spin* 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.	**D5001/D5002** (column) **D5003** (shallow-well plate) **D5004** (deep-well plate) **D5040** (magnetic bead)
EZ DNA Methylation-Gold™ Kit	For the fast (3 hr.) conversion of unmethylated cytosines in DNA to uracil via heat/chemical-denaturation of DNA and a specially designed CT Conversion Reagent. *Fast-Spin* 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.	**D5005/D5006** (column) **D5007** (shallow-well plate) **D5008** (deep-well plate) **D5042** (magnetic bead)
EZ DNA Methylation-Direct™ Kit	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.	**D5020/D5021** (column) **D5022** (shallow-well plate) **D5023** (deep-well plate) **D5044** (magnetic bead)
EZ DNA Methylation-Lightning™ Kit	Complete bisulfite conversion in about an hour using a unique liquid format conversion reagent. *Fast-Spin* 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.	**D5030/D5031** (column) **D5032** (shallow-well plate) **D5033** (deep-well plate) **D5046** (magnetic bead)
EZ DNA Methylation-Startup™ Kit	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	**D5024**
Methylated DNA Standards			
Universal Methylated Human DNA Standard	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	**D5011**
Universal Methylated Mouse DNA Standard	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	**D5012**
Region-Specific DNA Methylation Screening			
***OneStep* qMethyl™ Kit**	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.	**D5310** **D5311** (Lite)
***OneStep* qMethyl™ Array**	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.	**D5312**
Epigenetics Services *For more information, visit* http://www.zymoresearch.com/services *or inquire at* services@zymoresearch.com.			
Services for Methylated DNA Analysis 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.			
Services for Hydroxymethylated DNA Analysis 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.			
Hydroxymethylation Detection			
Quest 5-hmC™ DNA Enrichment Kit	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.	**D5420** **D5421**
Quest 5-hmC™ DNA ELISA Kit	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.	**D5425** **D5426**
Anti-5-Hydroxymethylcytosine Polyclonal Antibody	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	**A4001-50** **A4001-200**
DNA Degradase™ **DNA Degradase Plus™**	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	**E2016** **E2017** **E2020** **E2021**
Other…			
Zymo*Taq*™ DNA Polymerase	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	**E2001/E2001** (system) **E2003/E2004** (premix)
Methylated-DNA IP Kit	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.	**D5101**