ZYMO RESEARCH

DNA Purification Made Simple™

ZR-96 Genomic DNA Clean & Concentrator®-5

Clean and concentrate large-sized DNA from any enzymatic reaction or impure preparation.

Highlights

- 96-well plate recovery of large-sized DNA (*e.g.*, genomic, mitochondrial, plasmid (BAC/PAC), viral, phage, (wga) DNA, etc.) from any enzymatic reaction or impure preparation (*e.g.*, Proteinase K digestion). *No messy precipitations!*
- Unique spin column for low volume (≥15 µl) elution of ultra-pure, high-yield DNA.
- Eluted DNA is ideal for PCR, restriction endonuclease digestion, Sanger and Next-Gen sequencing, etc.

Catalog Numbers:
D4066, D4067

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

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Product Contents

ZR-96 Genomic DNA Clean & Concentrator®-5	D4066 (2 x 96 Preps.)	D4067 (4 x 96 Preps.)	Storage Temperature
ChIP DNA Binding Buffer	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-XL Plates	2	4	Room Temp.
Collection Plates	2	4	Room Temp.
Elution Plates	2	4	Room Temp.
Instruction Manual	1	1	-

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

Specifications

- **DNA Purity –** High-quality ($A_{(260/280)}$ ≥ 1.8) high molecular weight DNA ideal for ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.

- **DNA Size Limits –** Capable of purifying small DNA fragments >50 bp and large sized DNAs up to 200 kb.

- **DNA Recovery –** Typically, up to 5 µg total DNA per column can be eluted into as ≥15 µl of low salt **DNA Elution Buffer** or water.

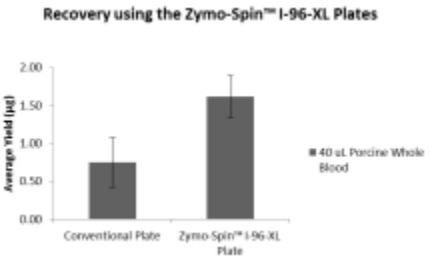
- **Sample Sources –** DNA from impure preparations of genomic DNA (*e.g.*, Proteinase K digestions), plasmid DNA (including BAC), viral DNA, and whole genome amplified (wga) DNA. Can also be used for the purification of low molecular weight DNA (50 bp to 10 kb) from PCR, endonuclease digestion, post-RT cDNA synthesis, etc. Suitable for isolated DNA stored in DNA/RNA Shield (page 6).

- **Product Detergent Tolerance –** ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 1% SDS.

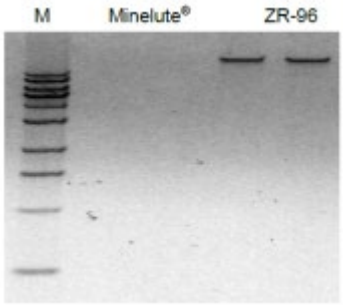
Product Description

The **ZR-96 Genomic DNA Clean & Concentrator®-5 (DCC®)** is for high throughput recovery of ultra-pure, large-sized DNA (*e.g.*, genomic, mitochondrial, plasmid (BAC/PAC), viral, phage, (wga) DNA, etc.) from any enzymatic reaction or impure preparation (*e.g.*, Proteinase K digestion). There is no need for organic denaturants, chloroform, or messy precipitations: simply add the specially formulated **ChIP DNA Binding Buffer** to a sample and then transfer the mixture to the supplied **Zymo-Spin™ I-96-XL Plate**. Eluted DNA is suitable for sequencing, PCR, endonuclease digestion, and other enzymatic procedures. The product is also compatible with smaller DNAs (50 bp to 10 kb) from PCR, digestions, crude plasmid preparations, cDNA synthesis, etc.

Simple **ZR-96 Genomic DCC®-5** workflow.



Zymo-Spin™ I-96-XL Plates result in superior yields to other conventional market columns. Genomic DNA extracted using the **Zymo-Spin™ I-96-XL Plate** results in higher yields from Porcine Whole Blood.



High molecular weight DNA is efficiently purified using the ZR-96 Genomic DCC®-25. Lambda (λ) phage DNA (48.5 kb) was purified (in duplicate) from input material using the Minelute® and the ZR-96 Genomic DCC®-5. The ZR-96 Genomic DCC®-5 resulted in yields > 340% compared to the Minelute®. Eluted DNAs were analyzed in a 0.8% (w/v) TAE/agarose/EtBr gel (shown above). The size marker "M" is a 1 kb ladder (Zymo Research).

Formats

	Genomic DCC™-10	Genomic DCC™-25	ZR-96 Genomic DCC™-5
			
Column	Zymo-Spin™ IC-XL	Zymo-Spin™ IIC-XL	Zymo-Spin™ I-96-XL
Capacity	10 µg/ prep.	25 µg/ prep.	5 µg/ prep.
Elution	≥ 10 µl	≥ 35 µl	≥ 15 µl

Applications

Post-PCR DNA Clean-up	Efficient desalting of DNA with the removal of DNA polymerases, primers, and free dNTPs.
DNA Clean-up From Enzymatic Reactions	Efficient desalting of DNA with the removal of modifying enzymes, RNA polymerases, ligases, kinases, nucleases, phosphatases, endonucleases, *etc.*
Plasmid DNA Clean-up	Efficiently purifies plasmid DNA from "home-made" preparations of cell free lysates or from commercial kits. Plasmid DNA purified and concentrated using the **Genomic DCC®** has proven an excellent substrate for high quality DNA sequencing.
Isotope and Dye Removal	Efficiently removes unincorporated fluorescent (*i.e.*, AMCA, FITC, BIO, DIG, Cy3, Cy5, FAM, *etc.*) and radiolabeled dNTP derivatives from DNA following *in vitro* labeling reactions.
Genomic DNA Clean-Up	Efficiently purifies genomic DNA from "home-made" preparations of cell free lysates or from commercial kits. Genomic DNA purified and concentrated using the **Genomic DCC®** has proven an excellent substrate for high quality DNA sequencing.

✓ For purification of DNA from 50 bp to 23 kb, use the **DNA Clean & Concentrator (D4003 & D4013).**

✓ For purification of short DNA or RNA oligonucleotides ≥ 16 nt, use the **Oligo Clean & Concentrator (D4060, D4061)**.

✓ For ChIP (Chromatin Immunoprecipitation) sample cleanup, use the **ChIP DNA Clean & Concentrator® (D5201, D5205)** for high quality DNA from any step in a standard ChIP protocol.

✓ For post-cycle sequencing samples, use the **ZR Sequencing DNA Clean-up Kit™ (D4050, D4051)** for dye blob elimination.

✓ For samples containing PCR inhibitors, use the ***OneStep*™ PCR Inhibitor Removal Kit (D6030, D6035)**.

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 3,500 – 5,000 x g.

1. Add 2-5 volumes of **ChIP DNA Binding Buffer** to each volume of DNA sample[1] (see table below). Mix thoroughly.

Application	DNA Binding Buffer : Sample	Example
Plasmid, genomic DNA (>2 kb)	2 : 1	200 µl : 100 µl
PCR product, DNA fragment	5 : 1	500 µl : 100 µl

2. Transfer sample mixtures to the wells of the provided **Zymo-Spin™ I-96-XL Plate**[2] mounted on a **Collection Plate**.

3. Centrifuge for 5 minutes. Discard the flow through.

4. Add 200 µl **DNA Wash Buffer** to each well. Centrifuge for 5 minutes. Repeat the wash step.

5. Transfer the **Zymo-Spin™ I-96-XL Plate** to an **Elution Plate**. Add ≥ 15 µl **DNA Elution Buffer**[3] or water[4] directly to the matrix of each well and incubate at room temperature for three minutes. Centrifuge for 5 minutes to elute the DNA.

   Ultra-pure DNA is now ready for use.

[1] It may be necessary to add RNase A to cell lysates prior to performing the procedure to ensure RNA-free DNA will be recovered in Step 5.
[2] The sample capacity is 900 µl/well. It may be necessary to load and spin a plate multiple times if a sample has a volume larger than 900 µl.
[3] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA
[4] Elution of DNA from the plate 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**.

Appendix

Isolated DNA stored in DNA/RNA Shield

*For previously isolated/purified DNA stored in **DNA/RNA Shield**, use the following protocol to recover ultra-pure DNA, ready for downstream applications.*

1. If frozen, thaw samples[1] at room temperature (20-30°C).

2. Add an equal volume of ethanol (95-100%) to the sample and mix well.

3. Continue with Step 2 of the Sample Processing Protocol on page 5.

RNase A Treatment

Dissolve RNase A (E1008-30), sold separately, in DNase/RNase-free water or TE to a stock concentration of 10 mg/ml.

1. Add enough 10 mg/ml RNase A to the sample for a final concentration of 10-100 µg/mL and mix well.

2. Incubate at room temperature for 15 minutes.

3. Continue with step 1 of the Sample Processing protocol on page 5.

[1] Adjust the sample volume to 50 µl (minimum) with **DNA/RNA Shield**.

Troubleshooting

Problem	Possible Causes and Suggested Solutions
Low Recovery	**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.
Following Clean-up with DCC®, 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
Genomic DNA Clean & Concentrator®-10 (for purification of up to 10 µg genomic DNA per prep.)	D4010 D4011	25 Preps. 100 Preps.
Genomic DNA Clean & Concentrator®-25 (for purification of up to 25 µg genomic DNA per prep.)	D4064 D4065	25 Preps. 100 Preps.
ZR-96 Genomic DNA Clean & Concentrator®-5 (for 96-well purification of up to 5 µg genomic DNA per well)	D4066 D4067	2 x 96 Preps. 4 x 96 Preps.

Individual Kit Components	Catalog No.	Amount
Chip DNA Binding Buffer	D5201-1-50 D5201-1-100	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	1 ml 4 ml
Collection Plates	C2002	2 Plates
Elution Plates	C2003	2 Plates
Zymo-Spin™ I-96-XL Plates	C2010-2 C2010-4	2 Plates 4 Plates

Complete Your DNA Methylation Workflow

✓ **Rapid Method for Complete Bisulfite Conversion of DNA**

EZ DNA Methylation Kits	Size	Catalog No.
EZ DNA Methylation-Lightning Kit	50 Rxns. 200 Rxns.	D5030 D5031
EZ-96 DNA Methylation-Lightning Kit	2x96 Rxns. (Deep-Well) 2x96 Rxns. (Shallow-Well)	D5032 D5033
EZ DNA Methylation-Lightning Automation Kit	96 Rxns.	D5049
EZ-96 DNA Methylation Lightning MagPrep	4 X 96 Rxns. 8 X 96 Rxns.	D5046 D5047

✓ **Innovative Solutions for Next Generation Sequencing**

Library Prep Kits	Size	Catalog No.
Zymo-Seq WGBS Library Kit	24 Preps.	D5465
Pico Methyl-Seq Library Prep Kit	10 Preps. 25 Preps.	D5455 D5456
Zymo-Seq RRBS Library Kit	24 Preps. 48 Preps.	D5460 D5461

✓ **Optimal Amplification of Bisulfite-Treated DNA**

ZymoTaq Polymerase	Size	Catalog No.
ZymoTaq Premix	50 Rxns. 200 Rxns.	E2003 E2004
ZymoTaq DNA Polymerase	50 Rxns. 200 Rxns.	E2001 E2002
ZymoTaq qPCR Premix	50 Rxns. 200 Rxns.	E2054 E2055

✓ **Industry Leading Tools for Assessing Your DNA Methylation Workflow**

DNA Methylation Standards	Size	Catalog No.
Human Methylated & Non-methylated DNA Set	5 µg/20 µl	D5014
Universal Methylated DNA Standard	Human Mouse	D5011 D5012
Bisulfite-Converted Universal Methylated Human DNA Standard	1 µg/50 µl	D5015
Human Methylated & Non-Methyated (WGA) DNA Set	5 µg/20 µl	D5013

Notes

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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.

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.

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