ZYMO RESEARCH

DNA Purification Made Simple™

DNA Clean & Concentrator®-500

High-quality DNA from samples including large-scale restriction endonuclease digestions and impure DNA preparations.

Highlights

- Simple, rapid recovery of ultra-pure DNA from large-scale sample sources.
- Unique column construction allows sample washing to be performed using a centrifuge or vacuum source.
- Eluted DNA is ideal for PCR, DNA sequencing, DNA transfection, DNA ligation, endonuclease digestion, RNA transcription, radiolabeling, *etc*.

Catalog Numbers:
D4031, D4032

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

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Table of Contents

Product Contents

DNA Clean & Concentrator®-500	D4031 (10 Preps.)	D4032 (20 Preps.)	Storage Temperature
DNA Binding Buffer	100 ml	2 x 100 ml	Room Temp.
DNA Wash Buffer[1]	24 ml	48 ml	Room Temp.
DNA Elution Buffer	2 x 16 ml	2 x 50 ml	Room Temp.
Zymo-Spin™ VI Columns	10	20	Room Temp.
Instruction Manual	1	1	-

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

Specifications

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

- **DNA Size Limits –** From ~50 bp to 23 kb.

- **DNA Recovery –** Typically, up to 500 µg total DNA per column can be eluted into as little as 2 ml 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 from enzymatic reactions (*e.g.*, PCR, restriction endonuclease digestions), plasmid preparations, and impure preparations. Suitable for isolated DNA stored in DNA/RNA Shield (page 8).

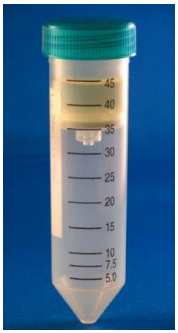
- **Product Detergent Tolerance –** ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS.

- **Equipment Needed –** Swinging bucket centrifuge or vacuum source (optional).

Product Description

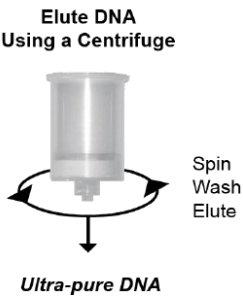
The **DNA Clean & Concentrator®-500 (DCC®-500)** is designed for the rapid, large format purification and concentration of up to 500 µg of high-quality DNA from samples including large-scale restriction endonuclease digestions and impure DNA preparations. The **DCC®-500** features a single-buffer system that allows for efficient DNA adsorption onto the matrix of the supplied **Zymo-Spin™ VI Column**. Simply add the specially formulated **DNA Binding Buffer** to your samples and transfer the mixtures to the supplied **Zymo-Spin™ VI Column**. The DNA is washed then eluted with a small volume (≥ 2 ml) of water or supplied **DNA Elution Buffer** using a centrifuge. The purified DNA is ideal for DNA ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures. The entire DNA purification/concentration procedure typically takes less than 20 minutes.

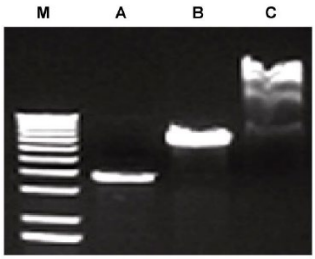
Loading and washing the Zymo-Spin™ VI Column can be performed using any combination of the following:



Centrifuge: Zymo-Spin™ VI Column inside a 50 ml conical tube.

Vacuum: Zymo-Spin VI™ Column connected to a vacuum manifold.





Ultra-pure DNA from the **DNA Clean & Concentrator** can be easily ligated for efficient cloning. Lanes: A: 3.5 kb blunt-ended vector; B: 7.4 kb blunt-ended DNA insert; C: Blunt-end ligation of A+B mixture.

Formats

	DCC™-5	DCC™-25	DCC™-100	DCC™-500	Genomic DCC™	ZR-96 DCC™-5
						
Name	Zymo-Spin™ I & IC	Zymo-Spin™ II & IIC	Zymo-Spin™ V	Zymo-Spin™ VI	Zymo-Spin ™ IC-XL	Zymo-Spin™ I-96
Capacity	5 µg/ prep.	25 µg/ prep.	100 µg/ prep.	500 µg/ prep.	10 µg/ prep.	5 µg/ prep.
Elution Vol.	≥ 6 µl	≥ 25 µl	≥ 150 µl	≥ 2 ml	≥ 10 µl	≥ 10 µl
Cat. Nos.	D4003, D4013	D4005, D4033	D4029, D4030	D4031, D4032	D4010, D4011	D4023, D4024

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.
Post-Reverse Transcription (RT) & cDNA Clean-up	Efficiently purifies DNA following RT, either as a DNA/RNA complex or as single stranded cDNA following chemical hydrolysis of the RNA template.
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 **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.
Purification of M13 ssDNA	The **DCC®** can be used for the rapid isolation of single stranded M13 phage DNA directly from phage-infected *E. coli* culture supernatant.

- ✓ 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.

Sample Processing

Add 2-7 volumes of **DNA Binding Buffer** to each volume of DNA sample (see table below). Mix briefly by gently inverting the tube.

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
ssDNA[2] (*e.g.,* cDNA, M13 phage)	7 : 1	700 µl : 100 µl

Use any of the following two procedures to process samples.

Centrifuge

1. Place the **Zymo-Spin™ VI Column** inside a 50 ml conical tube. Transfer the prepared sample mixture (see above) into the **Zymo-Spin™ VI Column**[3].

2. Centrifuge for 5 minutes at 3,000 x *g*. Discard the flow-through.

3. Add 10 ml **DNA Wash Buffer** to the **Zymo-Spin™ VI Column**. Centrifuge the **Zymo-Spin™ VI Column** for 5 minutes at 3,000 x *g*.

4. Transfer the **Zymo-Spin™ VI Column** into a new 50 ml conical tube. Add 2-3 ml **DNA Elution Buffer**[4] or water[5] directly to the column matrix. Wait for one minute to ensure that the column matrix has been fully hydrated. Centrifuge for 3 minutes at 3,000 x *g* to elute the DNA. Ultra-pure DNA is now ready for use.

[1] For efficient recovery of DNA > 20 kb, use the **Genomic DNA Clean & Concentrator (D4010, D4011)**.
[2] For ssDNA purification, see page 7 in the appendix.
[3] The sample capacity of the column is 15 ml. Therefore, it may be necessary to load a column multiple times if a sample has a volume larger than 15 ml.
[4] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM 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 an additional 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**.

Vacuum

1. Attach **Zymo-Spin™ VI Column** to a suitable vacuum manifold (see image on page 3). Transfer the prepared sample mixture (see previous page) into the **Zymo-Spin™ VI Column**[1].

2. Turn on vacuum source and wait for sample to clear from the column.

3. Add 5 ml **DNA Wash Buffer** to the **Zymo-Spin™ VI Column**. Repeat wash step. After washing, leave the vacuum source "on" for an additional 5 minutes to remove any residual **Wash Buffer** from the column.

4. Transfer the **Zymo-Spin™ VI Column** into a new 50 ml conical tube. Add 2-3 ml **DNA Elution Buffer**[2] or water[3] directly to the column matrix. Wait for one minute to ensure that the column matrix has been fully hydrated. Centrifuge at 3,000 x *g* for 3 minutes to elute the DNA. Ultra-pure DNA is now ready for use.

[1] The sample capacity of the column is 15 ml. Therefore, it may be necessary to load a column multiple times if a sample has a volume larger than 15 ml.
[2] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA
[3] Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. Waiting an additional 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**

Appendix

cDNA clean-up

*The **DCC®** kit can be used to effectively clean and concentrate cDNA (> 500 nt) following reverse transcription (RT) in the presence/absence of fluorescent dyes. Unincorporated free nucleotides and fluorescent derivatives are efficiently removed using the **DCC®**, and the recovered cDNA may be used directly for microarray analysis, second-strand cDNA synthesis, or indirect labeling with a fluorescent dye such as NHS ester Cy3 or Cy5.*

*For clean-up of short cDNAs or ESTs (≥ 16 nt), we recommend the **Oligo Clean & Concentrator (Cat. Nos. D4060, D4061)**.*

Hydrolysis

1. Add 10 µl 0.5 M EDTA and 10 µl 1 N NaOH to 50 µl of RT reaction.

   The volumes of EDTA and NaOH should be scaled proportionally depending on the starting volume of the RT reaction.

2. Incubate at 65°C for 15 minutes.

Clean-up

1. Add 490 µl (7 volumes) of **DNA Binding Buffer** to the hydrolysis reaction above. Mix well.

   Neutralization (pH) following RNA hydrolysis is not necessary as the **DNA Binding Buffer** will effectively neutralize the NaOH added to the reaction.

2. Continue with Step 1 of the Sample Processing Protocol on page 5 when using a centrifuge or page 6 when using a vacuum.

M13 phage ssDNA purification

1. Centrifuge phage-infected bacterial culture at 8,000 x *g* for 1 minute.

2. Transfer 100 µl of phage-containing supernatant to a 1.5 ml microcentrifuge tube and add 700 µl (7 volumes) of **DNA Binding Buffer**. Mix briefly by vortexing.

   Increased supernatant volumes may be processed by proportionally increasing the amount of **DNA Binding Buffer** added to the sample.

3. Continue with Step 1 of the Sample Processing Protocol on page 5 when using a centrifuge or page 6 when using a vacuum.

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 1 of the Sample Processing Protocol on page 5 when using a centrifuge or page 6 when using a vacuum.

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. Add the appropriate volume of DNA Binding Buffer using the table on page 5 in the Sample Processing Protocol and continue with Step 1 on page 5 when using a centrifuge or page 6 when using a vacuum.

[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
DNA Clean & Concentrator®-5 (for purification of up to 5 µg DNA per prep.) *Supplied with **uncapped columns***	D4003T D4003 D4004	10 Preps. 50 Preps. 200 Preps.
DNA Clean & Concentrator®-5 (for purification of up to 5 µg DNA per prep.) *Supplied with **capped columns***	D4013 D4014	50 Preps. 200 Preps.
ZR-96 DNA Clean & Concentrator®-5 (for 96-well purification of up to 5 µg DNA per well)	D4023 D4024	2 x 96 Preps. 4 x 96 Preps.
DNA Clean & Concentrator®-25 (for purification of up to 25 µg DNA per prep.) *Supplied with **uncapped columns***	D4005 D4006	50 Preps. 200 Preps.
DNA Clean & Concentrator®-25 (for purification of up to 25 µg DNA per prep.) *Supplied with **capped columns***	D4033 D4034	50 Preps. 200 Preps.
DNA Clean & Concentrator®-100 (for purification of up to 100 µg DNA per prep.)	D4029 D4030	25 Preps. 50 Preps.
DNA Clean & Concentrator®-500 (for purification of up to 500 µg DNA per prep.)	D4031 D4032	10 Preps. 20 Preps.

Individual Kit Components	Catalog No.	Amount
DNA Binding Buffer	D4003-1-L D4004-1-L	50 ml 100 ml
DNA Wash Buffer (concentrate)	D4003-2-24 D4003-2-48	24 ml 48 ml
DNA Elution Buffer	D3004-4-16 D3004-4-50	16 ml 50 ml
Zymo-Spin™ VI Columns	C1013-10 C1013-20	10 Pack 20 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 D4209 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
Zymo10B	10 x 100 µl aliquots 96 x 50 µl aliquots	T3019 T3020
JM109	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 ultra-pure DNA from agarose gels**

Zymoclean Gel DNA Recovery™	Size	Catalog No.
Zymoclean™ Gel DNA Recovery Kit	50 Preps. 200 Preps.	D4001 D4002

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.

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