DNA Clean & Concentrator™-5 Protocol

Zymo Research

Abstract

The **DNA Clean & Concentrator™-5 (DCC™-5)** provides a hassle-free method for the rapid purification and concentration of high-quality DNA from PCR, endonuclease digestions, cell lysates, and other impure DNA preparations. It can also be used for post-RT cDNA clean-up and purification of sequencing-ready DNA from M13 phage.

Citation: Zymo Research DNA Clean & Concentrator™-5 Protocol. **protocols.io**
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Guidelines

Product Contents

DNA Clean & Concentrator™-5 (Kit Size)	D4003, D4013 (50 Preps.)	D4004, D4014 (200 Preps.)	Storage Temperature
DNA Binding Buffer	50 ml	2 x 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™ Columns	50 D4003 – uncapped D4013 – capped	200 D4004 – uncapped D4014 – capped	Room Temp.
Collection Tubes	50	200	Room Temp.
Instruction Manual	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.

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

Specifications

- **DNA Purity** – High-quality DNA (A_{260}/A_{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 5 µg total DNA per column can be eluted into as little as 6 µl 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%.

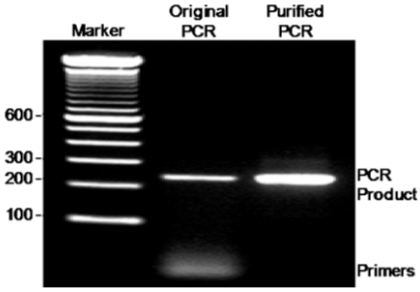
- **Sample Sources** – DNA from enzymatic reactions (e.g., PCR, restriction endonuclease digestions), plasmid preparations, and impure preparations.

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

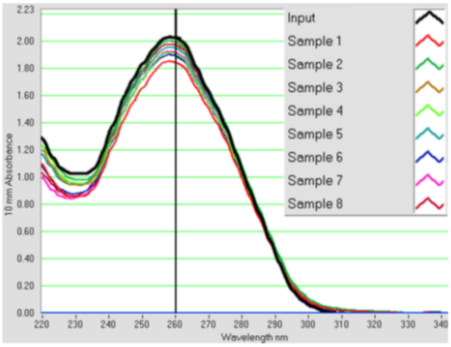
Product Description

The **DNA Clean & Concentrator™-5 (DCC™-5)** provides a hassle-free method for the rapid purification and concentration of high-quality DNA from PCR, endonuclease digestions, cell lysates, and other impure DNA preparations. It can also be used for post-RT cDNA clean-up and purification of sequencing-ready DNA from M13 phage. Simply add the specially formulated **DNA Binding Buffer** to your sample and transfer the mixture to the supplied **Zymo-Spin™ Column**. There is no need for organic denaturants or chloroform. Instead, the product features Fast-Spin column technology to yield DNA that is free of salts and contaminants in just 2 minutes. The purified DNA is ideal for DNA ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.

Two minute **DCC-5™** procedure.



DNA samples, such as the PCR products shown here, can be efficiently purified and concentrated using the **DNA Clean & Concentrator™-5**.



Pure and Reliable Recovery with the DCC™-5. Shown here is the recovery of 1 µg of 100 bp marker DNA eluted into 10 µl of water analyzed using a NanoDrop® spectrophotometer. The DNA Clean & Concentrator™-5 consistently recovers > 90% of input DNA.

Available 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

Typical DCC™ 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).**

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.

Troubleshooting

Low Recovery

- **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**

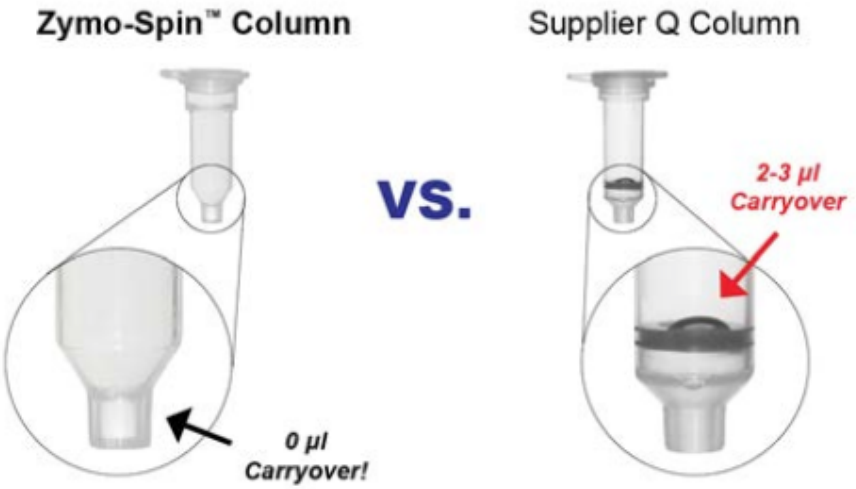
1. DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥ 50 kb), apply heated elution buffer (60-70 °C) to the column and incubate for several minutes prior to elution.

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 (see below).



Following Clean-up with the DCCTM, 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.

Selected Citations

Li, N. (2010). Whole genome DNA methylation analysis based on high throughput sequencing technology. Methods, 52 (3), 221-232.

Lee, EJ. (2011). Targeted bisulfite sequencing by solution selection and massively parallel sequencing. Nucleic Acids Research, 39(19),

e127, doi:10.1093/nar/gkr598

Papageorgiou, EA. (2009). Sites of differential DNA methylation between placenta and peripheral blood. Am J Pathol, 174 (5), 1609-1618.

Ferguson, A.A. et al. (2009). Retrofitting ampicillin resistant vectors by recombination for use in generating C. elegans transgenic animals by bombardment. Plasmid, 62, 140-145.

Before start

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.

Materials

DNA Clean & Concentrator™-5 D4003 by Zymo Research

Protocol

Step 1.
In a 1.5 ml microcentrifuge tube, add 2-7 volumes of DNA Binding Buffer to each volume of DNA sample (see table below). Mix briefly by vortexing.

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

For efficient recovery of genomic or large DNA (> 20 kb to > 200 kb), use the **Genomic DNA Clean & Concentrator™ (Cat. Nos. D4010, D4011).**

NOTES
Shea Biondi 25 Nov 2016

[1] For ssDNA purification, see Appendix A

Step 2.
Transfer mixture to a provided **Zymo-Spin™ Column**[2] in a **Collection Tube.**

NOTES
Shea Biondi 25 Nov 2016

[2] The sample capacity of the column is 800 µl. Therefore, it may be necessary to load and spin a column multiple times if a sample has a volume larger than 800 µl.

Step 3.
Centrifuge for 30 seconds. Discard the flow-through.

DURATION
00:00:30

Step 4.
Add 200 µl DNA Wash Buffer to the column. Centrifuge for 30 seconds. (wash 1/2)

DURATION
00:00:30

Step 5.
Add 200 µl DNA Wash Buffer to the column. Centrifuge for 30 seconds. (wash 2/2)

DURATION
00:00:30

Step 6.

Add ≥ 6 µl DNA Elution Buffer[3] or water[4] directly to the column matrix and incubate at room temperature for one minute.

DURATION
00:01:00

NOTES
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[3] **DNA Elution Buffer:** 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA

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[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.**

Step 7.
Transfer the column to a 1.5 ml microcentrifuge tube and centrifuge for 30 seconds to elute the DNA.

DURATION
00:00:30

NOTES
Shea Biondi 06 Dec 2016
Ultra-pure DNA is now ready for use.