ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

DNA Clean & Concentrator™-100

Catalog Nos. D4029 & D4030

Highlights

- Simple, rapid recovery of ultra-pure DNA from PCR, endonuclease digestions, and “crude” DNA preps., etc.
- Unique column construction allows sample washing to be performed using a centrifuge, microcentrifuge, vacuum source, or syringe.
- Column design allows DNA to be eluted at high concentrations into minimal volumes of water or TE buffer using a microcentrifuge.
- Eluted DNA is well suited for use in PCR, DNA sequencing, DNA ligation, endonuclease digestion, RNA transcription, radiolabeling, etc.

Contents

ZYMO RESEARCH CORP.

Toll Free: 1-888-882-9682 • Fax: 1-714-288-9643 • Web: www.zymoresearch.com • E-mail: info@zymoresearch.com

Product Contents

DNA Clean & Concentrator™-100 (Kit Size)	D4029 (25 preps.)	D4030 (50 preps.)	Storage Temperature
DNA Binding Buffer	100 ml	2 x 100 ml	Room Temp.
DNA Wash Buffer*	24 ml	48 ml	Room Temp.
Zymo-Spin™ V Columns	25 columns	50 columns	Room Temp.
Zymo Wash Columns (no filter)	25 columns	50 columns	Room Temp.
Collection Tubes	25 tubes	50 tubes	Room Temp.
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Note - 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 maximal performance and reliability.

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

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

Typical DNA Clean & Concentrator™ (DCC™) Applictions

- **Post-PCR DNA Clean-up**: Efficient desalting with the removal of DNA polymerases, primers, and free dNTPs.
- **DNA Clean-up from Enzymatic Reactions**: Efficient desalting with the removal of DNA and RNA polymerases, modifying enzymes, 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**: The DCC™ can be used to purify plasmid DNA from cell-free lysates from “home-made” preparations or from commercial kits. Plasmid DNA, purified and concentrated using the DCC™ has proven an excellent substrate for high quality sequencing.
- **Isotope and Dye Removal**: The DCC™ effectively removes unincorporated fluorescent (i.e., AMCA, FITC, BIO, DIG, Cy3, Cy5, FAME, Etc.) and radiolabeled dNTP derivatives from DNA following *in vitro* labeling reactions.
- **Purification of M13 ssDNA**: The DCC™ can be used for rapid isolation of single stranded M13 phage DNA directly from phage-infected *E.coli* culture supernatant.

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.

Product Description

The **DNA Clean & Concentrator™-100** (**DCC™-100**) was designed for the rapid purification and concentration of up to 100 µg of high quality DNA from PCR, large format restriction endonuclease digestions, or "crude" DNA preparations. The **DCC™-100** employs a single-buffer system that allows for efficient DNA adsorption onto the matrix of the supplied **Zymo-Spin™ V columns**. Simply add the specially formulated **DNA Binding Buffer** to your sample and transfer the mixture to the supplied **Zymo Wash Column** with an attached **Zymo-Spin™ V Column**. There is no need for organic denaturants or chloroform. DNA purified using the **DCC™-100** is suitable for nucleotide sequencing, array analysis, PCR, nucleotide blotting, restriction endonuclease digestion procedures, and many other downstream applications requiring high quality DNA.

The entire DNA purification/concentration procedure typically takes less than 20 minutes and can be performed using a syringe, centrifuge or vacuum source together with a microcentrifuge.

For **Technical Assistance**, please contact those at **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

Elute DNA using a microcentrifuge

Ultra-pure DNA for:

- Sequencing
- DNA Transfection
- Endonuclease Digestion
- Cloning
- Etc.

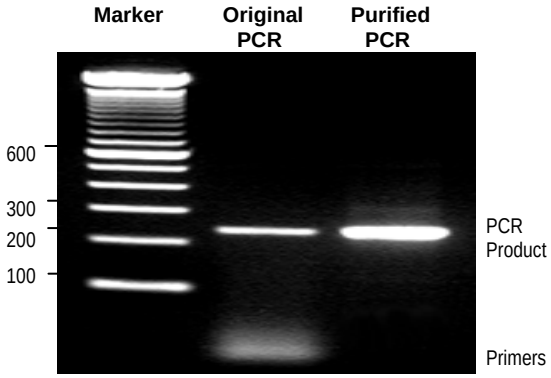

Figure 2. DNA samples, such as the PCR products shown here, can be efficiently purified and concentrated using the **DNA Clean & Concentrator™** product.

Specifications

- **Format:** Spin Column.
- **DNA Purity;** High-quality, purified DNA is eluted with water, which is especially well suited for sequencing, ligation reactions, and restriction endonuclease digestions.
- **DNA Size Limits;** From 75 bp to 23 kb.
- **DNA Recovery;** Typically, ≤ 100 µg total DNA can be eluted into ≥ 150 µl 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 from PCR, restriction endonuclease digestions, plasmid preparations, kinase reactions, etc.
- **Product Detergent Tolerance;** ≤5% Triton X-100, ≤5% Tween-20, ≤5% Sarkosyl, ≤0.1% SDS.
- **Equipment:** Microcentrifuge and centrifuge, vacuum source, or syringe.

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

Centrifuge: Column assembly (**Zymo-Spin™ V Column** attached to the bottom of the **Zymo Wash Column**) inside a 50 ml conical tube. Appropriate for total volumes of ≥ 600 µl.

Microcentrifuge: Zymo-Spin™ V Column placed inside a **Collection Tube.** Appropriate for total volumes of ≤ 600 µl.

Vacuum: Column assembly (**Zymo-Spin™ V Column** attached to the bottom of the **Zymo Wash Column**) connected to a vacuum manifold. Appropriate for total volumes of ≥ 600 µl.

Syringe: Zymo-Spin™ V Column attached to the tip of a syringe. Appropriate for total volumes of ≥ 600 µl.

Reagent Preparation

Before starting, add 192 ml 100% ethanol to the 48 ml **DNA Wash Buffer** concentrate to obtain the final **DNA Wash Buffer** solution.

Note: Alternatively, add 104 ml 95% ethanol to the 24 ml size of the **DNA Wash Buffer** concentrate.

Microcentrifuge Protocol

1. In a 1.5 ml microcentrifuge tube, add two volumes of **DNA Binding Buffer** to each volume of DNA sample (e.g., 400 µl **DNA Binding Buffer** to 200 µl DNA sample). Mix briefly by gently inverting the tube and then add the sample mixture to the **Zymo-Spin™ V Column** placed inside a **Collection Tube.**

2. Centrifuge at maximum speed (10,000 – 14,000 rpm) for 1 minute. Discard the flow-through.

3. Add 600 µl **Wash Buffer** to the **Zymo-Spin™ V Column**. Centrifuge at maximum speed for 1 minute. Discard the flow-through and repeat wash step.

4. Transfer the **Zymo-Spin™ V Column** into a new **Collection Tube** and "quick spin" for 30 seconds at (10,000 – 14,000 rpm) to remove any residual **Wash Buffer**.

5. Transfer the **Zymo-Spin™ V Column** into a new 1.5 ml microcentrifuge tube. Add 150 µl water or low salt elution buffer directly to the column matrix in the **Zymo-Spin™ V Column.** Wait for one minute to ensure that the column matrix has been fully hydrated prior to centrifugation at (10,000 – 14,000 rpm) for 1 minute to elute DNA.

   Ultra-pure, concentrated DNA in water is now ready for use.

Note: For ssDNA (e.g., single stranded cDNA), add 7 volumes **DNA Binding Buffer** to each volume of sample. (For example, 350 µl **DNA Binding Buffer** to a 50 µl sample.)

Note: Minimal elution volume of 150 µl results in a highly concentrated DNA sample. If a larger volume is desired, more water or elution buffer can be used for a less concentrated sample.

Centrifuge Protocol

1. In a tube, add two volumes of **DNA Binding Buffer** to each volume of DNA sample (e.g., 2 ml **DNA Binding Buffer** to 1 ml DNA sample). Mix briefly by gently inverting the tube and then add the sample mixture to the **Zymo Wash Column/Zymo-Spin™ V Column assembly** inside a 50 ml conical tube**.**

2. Centrifuge at 4,000 rpm (~ 3,000 x *g*) for 5 minutes. Discard flow-through.

3. Add 2 ml **Wash Buffer** to the **Zymo Wash Column**. Centrifuge at ≥ 3,000 x *g* for 5 minutes. Discard flow-through. Repeat wash step. (Alternatively, one wash can be performed using 4 ml **Wash Buffer**).

4. Transfer the **Zymo-Spin™ V Column** into a new 1.5 ml microcentrifuge tube. Add 150 µl water or low salt elution buffer directly to the column matrix in the **Zymo-Spin™ V Column.** Wait for one minute to ensure that the column matrix has been fully hydrated prior to centrifugation at (10,000 – 14,000 rpm) for 1 minute to elute DNA.

   Ultra-pure, concentrated DNA in water is now ready for use.

Note: Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >5.0. Waiting 1 minute after adding water to the column 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 water.

Note: TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or modified TE (10 mM Tris, 0.1 mM EDTA, pH 8.5) can also be used for elution if required by your experiment.

Vacuum/Syringe Protocol

1. **Vacuum**: Attach the **Zymo-Spin™ V Column** to the bottom of the **Zymo Wash Column** and then connect the column assembly to a suitable vacuum manifold (see illustration on page 3). In a separate tube, add two volumes of **DNA Binding Buffer** to each volume of DNA sample. Mix briefly by inverting gently. Then, pour the mixture into the **Zymo Wash Column**. Turn on the vacuum source until the entire mixture has passed through the **Zymo-Spin™ V Column**.

   Alternative method using a syringe: Add two volumes of **DNA Binding Buffe**r to each volume of DNA sample. Mix briefly by inverting. Then collect the mixture using a syringe. Once all of the mixture has been drawn inside the syringe, attach a **Zymo-Spin™ V Column** and push the entire mixture out of the syringe through the column.

2. **Vacuum**: Add 2 ml **Wash Buffer** to the **Zymo Wash Column** attached to a **Zymo-Spin™ V Column**. Turn on the vacuum source until all of the mixture has passed through the **Zymo Spin™ V Column**. Repeat wash step. (Alternatively, one wash can be performed using 4 ml **Wash Buffer**). After washing, leave the vacuum source "on" for an additional 5 minutes to remove any residual wash buffer from the column.

   Alternative method using a syringe: Aspirate 2 ml **Wash Buffer** using a clean syringe, re-attach the **Zymo-Spin™ V Column** and push the **Wash Buffer** out of the syringe through the column. Repeat wash step. (Alternatively, one wash can be performed using 4 ml **Wash Buffer**). It's recommended to "quick spin" the **Zymo-Spin™ V Column** (in a collection tube) after the last wash to completely remove any residual wash buffer.

5. Transfer the **Zymo-Spin™ V Column** into a new 1.5 ml microcentrifuge tube. Add 150 µl water or low salt elution buffer directly to the column matrix in the **Zymo-Spin™ V Column.** Wait for one minute to ensure that the column matrix has been fully hydrated prior to centrifugation at (10,000 – 14,000 rpm) for 1 minute to elute DNA.

   Ultra-pure, concentrated DNA in water is now ready for use.

Note: For ssDNA (e.g., single stranded cDNA), add 7 volumes to each volume of sample. For example, 350 µl **DNA Binding Buffer** to a 50 µl sample.

Note: Minimal elution volume of 100 µl results in a highly concentrated DNA sample. If a larger volume is desired, more water or elution buffer can be used for a less concentrated sample.

Note: Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >5.0. Waiting 1 minute after adding water to the column 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 water.

Note: TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or modified TE (10 mM Tris, 0.1 mM EDTA, pH 8.5) can also be used for elution if required by your experiment.

Helpful Hints

For your convenience, sample loading and washing can be performed using any combination of the methods outlined on page 3. However, if dealing with large sample volumes it may be necessary to use centrifugation, vacuum, or syringe-based procedures. For the highest attainable DNA yields, it is recommended to use a microcentrifuge when eluting DNA.

Ordering Information

Product Description	Catalog No.	Kit Size
DNA Clean & Concentrator™-100	D4029	25 preps.
DNA Clean & Concentrator™-100	D4030	50 preps.

For Individual Sale	Catalog No.	Amount
DNA Binding Buffer	D4004-1-L	100 ml
DNA Wash Buffer (concentrate)	D4003-2-24 D4003-2-48	24 ml 48 ml
Zymo-Spin™ V Columns	C1012-25-25	25 columns

Popular DNA Purification & Analysis Products from Zymo

Product	Description	Kit Size	Cat No. (Column Format)
DNA Clean & Concentrator™-5	Clean and concentrate DNA from any reaction or "crude" preparation in 2 min. A 6 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 5 µg of DNA.	50 Preps 200 Preps 50 Preps 200 Preps	**D4003** (uncapped) **D4004** (uncapped) **D4013** (capped) **D4014** (capped)
ZR-96 DNA Clean & Concentrator™-5	Quick (15 mins), high-output recovery of pure DNA from PCR, endonuclease digestions, plasmid preparations, etc. 10-15 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 5 µg of DNA.	2x96 Preps 4x96 Preps	**D4023** **D4024**
DNA Clean & Concentrator™-25	Clean and concentrate DNA from any reaction in 2 min. 25 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 25 µg of DNA.	50 Preps 200 Preps	**D4005** **D4006**
Zymoclean Gel DNA Recovery Kit™	Purify DNA from agarose gels in as little as 15 min. DNA sizes range from 75 bp to 23 Kb. 6 µl minimal elution volume (70-95% recovery rate).	50 Preps 200 Preps	**D4001** **D4002**
ZR-96 Zymoclean Gel DNA Recovery Kit™	High-output DNA purification from agarose gels in as little as 15 min. DNA sizes range from 75 bp to 23 Kb.	2x96 Preps 4x96 Preps	**D4021** **D4022**
EZ DNA Methylation Kit™	Streamlined kit for the conversion of unmethylated cytosines in DNA to uracil via the chemical-denaturation of DNA and a specially designed CT Conversion Reagent™. DNA is then desulphonated and subsequently cleaned using Fast-Spin column technology. No messy precipitations. Ultra-pure recovered DNA can be used for Methylation Specific PCR (MSP) and bisulfite sequencing applications.	50 Rxns 200 Rxns 2x96 Rxns	**D5001** **D5002** **D5003**
EZ DNA Methylation-Gold Kit™	Streamlined kit for the conversion of unmethylated cytosines in DNA to uracil via heat-denaturation of DNA and a specially designed CT Conversion Reagent™. DNA is then desulphonated and subsequently cleaned using Fast-Spin column technology. No messy precipitations. Ultra-pure recovered DNA can be used for Methylation Specific PCR (MSP) and bisulfite sequencing applications.	50 Rxns 200 Rxns 2x96 Rxns	**D5005** **D5006** **D5007**
Pinpoint Slide DNA Isolation System™	Recover genomic DNA from paraffin-embedded or fresh tissue sections for PCR. Ideal for isolating DNA from clinical tissue samples.	50 Preps	**D3001**
ZR Genomic DNA I Kit™	Genomic DNA isolation from whole blood, tissue culture cells, solid tissue and liquid samples. (Silica bead format is scalable to fit your requirements).	100 Preps 400 Preps	**D3004** **D3005**
ZR Genomic DNA II Kit™	Genomic DNA purification from whole blood, tissue culture cells, solid tissue and liquid samples. No requirement for beads or phenol chloroform.	50 Preps 200 Preps	**D3006** **D3007**
ZR-96 Genomic DNA Kit™	High-output genomic DNA purification from whole blood, tissue culture cells, solid tissue and liquid samples. No requirement for beads or phenol chloroform.	2x96 Preps 4x96 Preps	**D3010** **D3011**
Zymoprep I™	Efficient purification method for yeast plasmid DNA: Works well for library plasmid recovery from yeast two-hybrid screens.	100 Preps	**D2001**
Zymoprep II™	Efficient purification method for yeast plasmid DNA: Works well for library plasmid recovery from yeast two-hybrid screens.	50 Preps	**D2004**
Zyppy Plasmid Miniprep I Kit™	High-purity plasmid DNA purification in minutes: (alkaline lysis/Fast-Spin column format for low elution volume 40 µl).	100 Preps 400 Preps	**D4015** **D4016**
Zyppy Plasmid Miniprep II Kit™	High-purity plasmid DNA purification in minutes: (alkaline lysis/Fast-Spin column format for low elution volume 25 µl).	100 Preps 400 Preps	**D4019** **D4020**
ZR Viral DNA Kit™	Isolation of viral DNA from cell-free body fluids or sample mixtures containing cells at concentrations less than 10^5 cells per ml.	50 Preps 200 Preps	**D3015** **D3016**
ZR-96 Viral DNA Kit™	High-output (96-well) isolation of viral DNA from cell-free body fluids or sample mixtures containing cells at concentrations less than 10^5 cells per ml.	2x96 Preps 4x96 Preps	**D3017** **D3018**

**Bulk quantities are available upon request. Please contact: busdev@zymoresearch.com or call 1-888-882-9682 for assistance.*