ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR-96 ChIP DNA Clean & Concentrator™

Catalog Nos. **D5206 & D5207**

Highlights

- Rapid high throughput (96-well) recovery of ultra-pure DNA from chromatin immunoprecipitation (ChIP), cell lysates, Proteinase K digested samples, PCRs, and other enzymatic reactions.
- Plate design permits DNA elution at high concentrations into minimal volumes (≥10 µl/well).
- Omits the use of organic solvents and the need for ethanol precipitation.
- Eluted DNA is well suited for use in PCR, Next-Gen sequencing (ChIP-Seq), microarrays, Southern blot analysis, and other molecular applications.

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ZYMO RESEARCH CORP.

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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 ChIP DCC™ (Kit Size)	D5206 (2 x 96 Preps.)	D5207 (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	4 ml	10 ml	Room Temp.
Zymo-Spin™ I-96 Plate	2	4	Room Temp.
Collection Plate	2	4	Room Temp.
Elution Plate	2	4	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 the **DNA Wash Buffer** label.

Specifications

- **DNA Purity** – High-quality, purified DNA is eluted with elution buffer or water and is especially well suited for PCR, Next-Gen sequencing (ChIP-Seq), microarrays, Southern blot analysis, and other molecular applications.
- **DNA Size Limits** – From ~50 bp to 23 kb.
- **DNA Recovery** – Typically, up to 5 µg total DNA (per well) can be eluted into as little as 10 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** – Any step in a standard ChIP protocol including:

    a) Samples that have undergone reverse cross-linking and Proteinase K or RNase A digestion following mechanical or enzymatically-mediated DNA shearing.

    b) Reverse cross-linked samples eluted from chromatin-antibody-bead complexes in TES, 0.1M $NaHCO_3$ and 1% SDS, or other buffers containing up to 1% SDS.

    Note: This kit can also be used for DNA purification from PCR, restriction digests, kinase, phosphatase and other enzymatic reactions.

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

Product Description

Chromatin immunoprecipitation (ChIP) is a powerful tool employed for the identification of nuclear proteins, such as histones and transcription factors, which are associated with specific regions of genomic DNA. ChIP has quickly become the principle technique for studying transcriptional regulation because it enables scientists to assess where gene regulatory proteins interact in the genome and to ascertain if a specific genomic locus has undergone histone modification.

The ChIP procedure involves formaldehyde-mediated covalent protein-DNA cross-linking followed by cell lysis and DNA shearing. An antibody specific for the protein of interest is typically used in conjunction with either Protein A or G agarose beads to immunoprecipitate the protein-DNA complexes. Following a reverse cross-linking procedure and Proteinase K digestion, the DNA is isolated for analysis.

The **ZR-96 ChIP DNA Clean & Concentrator™** (**ZR-96 ChIP DCC™**) provides a hassle-free method for the rapid, high throughput purification and concentration of high-quality DNA from any step in a "standard" ChIP protocol. This includes samples that have: A) undergone reverse cross-linking and Proteinase K or RNase A digestion following mechanical/enzymatically-mediated DNA shearing or B) reverse cross-linked samples eluted from chromatin-antibody-bead complexes. *Additionally, this product may also be used to purify DNA from PCR and other enzymatic reactions.* DNA purified using the **ZR-96 ChIP DCC™** is suitable for PCR, Next-Gen sequencing (ChIP-Seq), arrays, as well as other molecular applications.

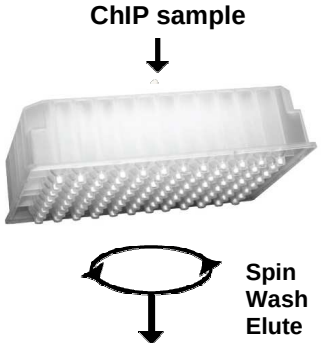

Figure 1: **ZR-96 ChIP DNA Clean & Concentrator™ procedure**. The **ZR-96 ChIP DCC™** employs a single buffer system that allows for efficient DNA adsorption to the matrix of the supplied **Zymo-Spin™ I-96 Plate**. The DNA is washed twice then eluted with a small volume of elution buffer or water.

Ultra-pure DNA is ideal for…

- ✓ PCR Analysis
- ✓ Next-Gen Sequencing
- ✓ Microarrays

The **ZR-96 ChIP DNA Clean & Concentrator™** can be used to recover ultra-pure DNA from cell lysates in a quantitative manner (Figure 2) and can also recover pure DNA from the eluates of chromatin-antibody-bead complexes following reverse cross-linking (Figure 3).

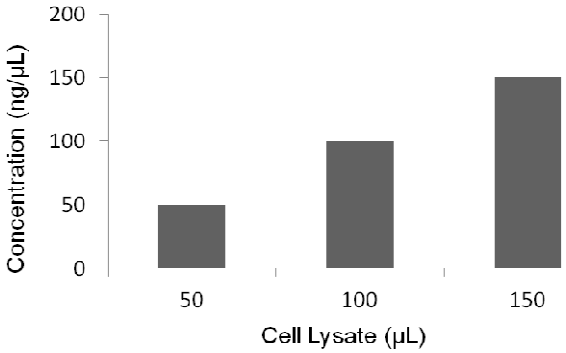

Figure 2: Quantitative recovery of DNA from cell lysates. The ZR-96 ChIP DNA Clean & Concentrator™ was used to purify DNA from lysates. The amount of DNA recovered was proportional to the lysate volume. Ultra-pure DNA isolated from 50, 100, and 150 µl cell lysates was eluted with 10 µl elution buffer and the DNA concentrations were determined using UV-Vis spectrophotometery.

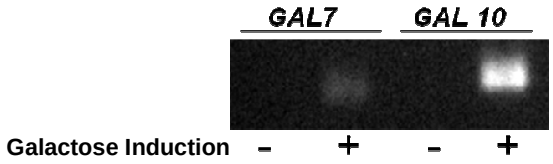

Figure 3: Yeast ChIP PCR Analysis. *S. cerevisiae* cultures were incubated at 30°C for 45 min. in YEP with or without galactose. Following cross-linking, cell lysis, and DNA shearing, ChIP was performed using an antibody specific for RNA polymerase II. Reverse cross-linking was followed by Proteinase K digestion and DNA purification using the ChIP DNA Clean and Concentrator™. PCR was performed using primers to *GAL* regions and the products were subsequently analyzed by agarose gel electrophoresis.

Available Formats

	ChIP DCC™	ChIP DCC™	ZR-96 ChIP DCC™
			
Name	Zymo-Spin™ I	Zymo-Spin™ IC	Zymo-Spin™ I-96
Capacity	5 µg/prep.	5 µg/prep.	5 µg/well
Elution Vol.	≥ 6 µl	≥ 6 µl	≥ 10 µl
Cat. Nos.	D5201	D5205	D5206, D5207

Selected References

Pehkonen, P et al. (2012). Genome-wide landscape of liver X receptor chromatin binding and gene regulation in human macrophages. *BMC Genomics, 13:50.*

Gong, M et al. (2011). KLF6/Sp1 initiates transcription of the *tmsg*-1 gene in human prostate carcinoma cells: An exon involved mechanism. *Journal of Cellular Biochemistry, 113:329-339.*

DiNatale, BC et al. (2010). Mechanistic Insights into the Events That Lead to Synergistic Induction of Interleukin 6 Transcription upon Activation of the Aryl Hydrocarbon Receptor and Inflammatory Signaling. *Journal of Biological Chemistry, 285:24388-24397*.

Buffer Preparation

✓ *Before starting:* Add 96 ml 100% ethanol (or 104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate. Add 192 ml 100% ethanol (or 208 ml 95% ethanol) to the 48 ml **DNA Wash Buffer** concentrate.

Protocol

Note: All centrifugation steps should be performed between 3,000 - 5,000 x g.

1. Add 5 volumes of **ChIP DNA Binding Buffer**[1] to each volume of DNA sample. Mix briefly by vortexing.

   Example: Add 250 µl ChIP DNA Binding Buffer to 50 µl eluent in TES or 0.1M $NaHCO_3$ containing 1% SDS buffers from chromatin-antibody-Protein A agarose-bead complexes followed by reverse cross-linking and Proteinase K digestion.

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

3. Centrifuge for 5 minutes until sample mixtures have been completely filtered. Discard the flow-through.

4. Add 300 µl **DNA Wash Buffer** to each well of the **Zymo-Spin™ I-96 Plate**. Centrifuge for 5 minutes. Repeat wash step, but centrifuge for 15 minutes.

5. Add ≥ 10 µl **DNA Elution Buffer**[3,4] 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 5 minutes to elute the DNA.

   Ultra-pure DNA is now ready for use in PCR, Next-Gen sequencing (ChIP-Seq), microarrays, and other applications.

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

Notes:

[1] Add 100 µl **ChIP DNA Binding Buffer** to all samples ≤ 20 µl.

[2] The capacity of each well of the **Zymo-Spin™ I-96 Plate** is approximately 1.1 ml. The capacity of each well of the **Collection Plate** is approximately 800 µl. Therefore, it may be necessary to load and spin the plate multiple times if a sample has a volume larger than 800 µl.

[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 > 5.0.

Ordering Information

Product Description	Catalog No.	Kit Size (Preps.)
ChIP DNA Clean & Concentrator™ (for purification of up to 5 µg DNA per prep.) *Supplied with **uncapped columns***	D5201	50
ChIP DNA Clean & Concentrator™ (for purification of up to 5 µg DNA per prep.) *Supplied with **capped columns***	D5205	50
ZR-96 ChIP DNA Clean & Concentrator™ (for 96-well purification of up to 5 µg DNA per well)	D5206 D5207	2 x 96 4 x 96

Refer to page 3 to see kit differences in column design.

For Individual Sale	Catalog No.	Amount
ChIP DNA Binding Buffer	D5201-1-50 D5201-1-100	50 ml 100 ml
DNA Wash Buffer (concentrate)	D4003-2-24 D4003-2-48	24 ml 48 ml
DNA Elution Buffer	D3004-4-1 D3004-4-4	1 ml 4 ml
Zymo-Spin™ I-96 Plate	C2004	2 plates
Collection Plate	C2002	2 plates
Elution Plate	C2003	2 plates

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Epigenetics Products From Zymo Research

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**