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ZYMO RESEARCH

DNA
Purification
FAST • EASY • SURE
Make Simple

ZR-96 ChIP DNA Clean & Concentrator[®]

DNA clean-up from any step in a standard ChIP protocol.

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.

Catalog Numbers:
D5206, D5207



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



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Product Contents

ZR-96 ChIP DCC®	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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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 NaHCO₃ 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.

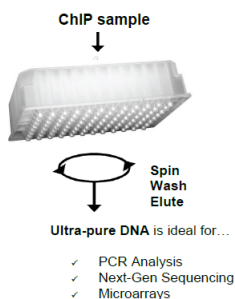


Figure 1: ZR-96 ChIP DNA Clean & Concentrator[®] procedure. The ZR-96 ChIP DCC[®] employs a single buffer system that allows for efficient DNA adsorption to the matrix of the supplied Zymo-Spin[™] I-96 Plate. The DNA is washed twice then eluted with a small volume of elution buffer or water.

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).

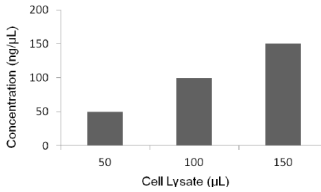


Figure 2: Quantitative recovery of DNA from cell lysates. The ZR-96 ChIP DNA Clean & Concentrator[®] was used to purify DNA from lysates. The amount of DNA recovered was proportional to the lysate volume. Ultra-pure DNA isolated from 50, 100, and 150 μl cell lysates was eluted with 10 μl elution buffer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

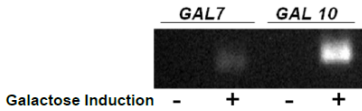





Figure 3: Yeast ChIP PCR Analysis. *S. cerevisiae* cultures were incubated at 30°C for 45 min. in YEP with or without galactose. Following cross-linking, cell lysis, and DNA shearing, ChIP was performed using an antibody specific for RNA polymerase II. Reverse cross-linking was followed by Proteinase 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.

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.

Protocol

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.

Sample Processing

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 NaHCO₃ 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 water⁴ 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.

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.	Size
ChIP DNA Clean & Concentrator[®] (for purification of up to 5 µg DNA per prep.) <i>Supplied with uncapped columns</i>	D5201	50 Preps.
ChIP DNA Clean & Concentrator[®] (for purification of up to 5 µg DNA per prep.) <i>Supplied with capped columns</i>	D5205	50 Preps.
ZR-96 ChIP DNA Clean & Concentrator[®] (for 96-well purification of up to 5 µg DNA per well)	D5206 D5207	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)	D4003-2-24 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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