ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ChIP DNA Clean & Concentrator™

Catalog Nos. **D5201 & D5205**

Highlights

- Quick (2 minute) recovery of ultra-pure DNA from chromatin immunoprecipitation (ChIP) assays, cell lysates, Proteinase K digested samples, PCRs and other enzymatic reactions.
- Column design allows DNA to be eluted at high concentrations into minimal volumes (≥ 6 µl) of water or low salt buffer.
- Eluted DNA is ideal for PCR amplification, arrays, DNA quantification, Southern blot analysis, and other molecular applications.
- Omits the use of organic solvents and the need for ethanol precipitation.

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ZYMO RESEARCH CORP.

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

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

Product Contents

ChIP DNA Clean & Concentrator™ (Kit Size)	D5201 (50 Preps.)	D5205 (50 Preps.)	Storage Temperature
ChIP DNA Binding Buffer	50 ml	50 ml	Room Temp.
DNA Wash Buffer*	6 ml	6 ml	Room Temp.
Elution Buffer	10 ml	10 ml	Room Temp.
Zymo-Spin™ Columns**	50 columns Uncapped columns	50 columns Capped columns	Room Temp.
Collection Tubes	50 tubes	50 tubes	Room Temp.
Instruction Manual	1	1	-

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 the highest performance and reliability.

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

** **D5201** contains uncapped Zymo-Spin™ I columns and **D5205** contains capped Zymo-Spin™ IC columns.

Specifications

- **DNA Purity** – High quality, purified DNA is eluted with elution buffer or water and is especially well suited for PCR amplification, arrays, Southern blot analysis, DNA quantification, sequencing, and other molecular applications.
- **DNA Size Limits** – From 50 bp to ~23 kb.
- **DNA Recovery** – Typically, up to 5 µg total DNA can be eluted from the spin column in as little as 6 µl 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** – Wherever DNA isolation and purification is required during standard ChIP protocols. This includes samples that have undergone reverse cross-linking and Proteinase K or RNase A digestion following either 1) mechanical or nuclease-mediated DNA shearing or 2) elution from chromatin-antibody-bead complexes in TES, 0.1M $NaHCO_3$ and 1% SDS, or other buffers containing up to 1% SDS. This kit can also be used for DNA purification from PCR, enzymatic digestion, kinase, phosphatase and other enzymatic reactions.
- **Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 1% SDS.

 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

Chromatin immunoprecipitation (ChIP) is a powerful tool employed for the identification of nuclear proteins, such as histones and transcription factors that are associated with specific regions of genomic DNA. ChIP has quickly become the principle technique for studying transcriptional regulation for 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 **Chromatin Immunoprecipitation (ChIP) DNA Clean & Concentrator™** provides a hassle-free method for the rapid purification and concentration of high quality DNA from any step in a "standard" ChIP protocol. This includes samples that have undergone reverse cross-linking, Proteinase K or RNase A digestion, mechanical or nuclease-mediated DNA shearing, and samples eluted from chromatin-antibody-bead complexes. *Additionally, this product may also be used to purify DNA from PCR and other enzymatic reactions.*

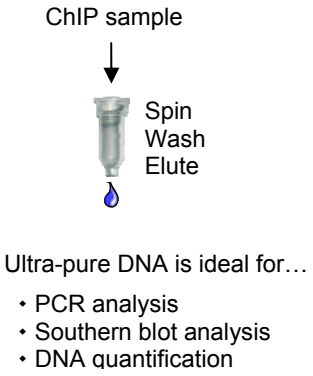

Figure 1: Two minute ChIP DNA Clean & Concentrator™ procedure. The ChIP DNA Clean & Concentrator™ employs a single buffer system that allows for efficient DNA adsorption to the matrix of the supplied Zymo-Spin™ Column. The DNA is washed twice then eluted with a small volume of elution buffer or water. The entire DNA purification/concentration procedure typically takes about 2 minutes.

The specially formulated **ChIP DNA Binding Buffer** promotes DNA adsorption to the column in the presence of detergents, antibodies, and proteinases that are often used for ChIP. Simply add the **ChIP DNA Binding Buffer** to your sample and transfer the mixture to the supplied **Zymo-Spin™ Column**. There is no need for organic extraction or ethanol precipitation. Instead, *Fast-Spin* column technology yields ultra-pure DNA in just minutes. The DNA purified using the **ChIP DNA Clean & Concentrator™** is ideal for PCR amplification, arrays, DNA quantification, as well as other molecular applications. This kit may be applied to any routine ChIP procedure to determine DNA concentration of samples that have undergone reverse cross-linking following DNA shearing. It can also be used for the removal of TES, 0.1M $NaHCO_3$, and 1% SDS from DNA eluted from chromatin-antibody-bead complexes and can be used to purify DNA from buffers containing up to 1% SDS or 5% NP-40, Tween-20, Triton X-100 or Sarkosyl.

The **ChIP DNA Clean & Concentrator™** recovers ultra-pure DNA from cell lysates that is proportional to the lysate volume and DNA fragment size range (see Figures 2 and 3 below). In these experiments, sample preparation was performed according to standard ChIP protocols where formaldehyde was used for protein-DNA cross-linking after cell lysis and DNA shearing. Protein-DNA complexes were then reverse cross-linked, treated with Proteinase K, and the DNA purified using the **ChIP DNA Clean & Concentrator™**.

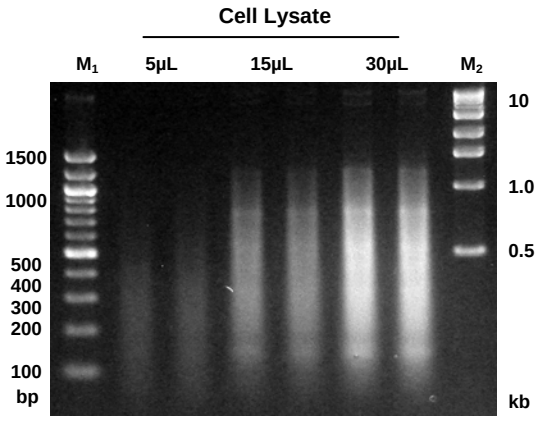

Figure 2: Agarose gel electrophoresis of DNA isolated from cell lysates. High quality DNA can be efficiently recovered from *Saccharomyces cerevisiae* cell lysates using the **ChIP DNA Clean and Concentrator™**. Duplicate purifications were performed with 5, 15 and 30 µl cell lysate and an equal volume of eluted DNA was loaded into each lane. The size marker M_1 and M_2 are 100 bp and 1 kb ladders, respectively (Zymo Research).

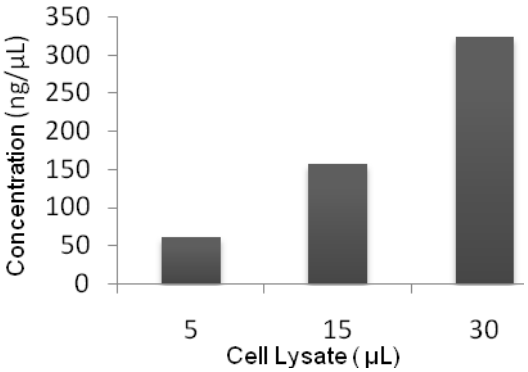

Figure 3: Quantitative recovery of DNA from cell lysates. The **ChIP DNA Clean & Concentrator™** was used to purify DNA from lysates, and the amount of DNA recovered was proportional to the lysate volume. Ultra-pure DNA isolated from 5, 15 and 30 µl cell lysates was eluted with 10 µl elution buffer and the DNA concentrations were determined using UV spectrophotometery.

The **ChIP DNA Clean & Concentrator™** can also recover pure DNA from the eluates of chromatin-antibody-bead complexes following reverse cross-linking and Proteinase K digestion in TES buffer. Figure 4 shows the results of PCR using DNA recovered by the product following ChIP with yeast cell lysates. This experiment demonstrates RNA polymerase II to be strongly associated with *GAL7* and *GAL10* chromatin fragments following induction of *GAL* genes in yeast cells.

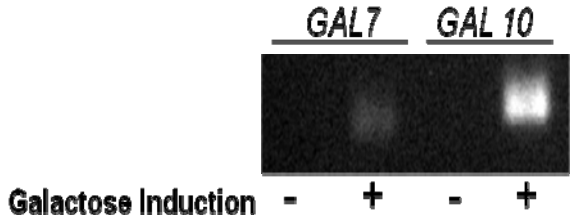

Figure 4: Yeast ChIP PCR Analysis. *Saccharomyces cerevisiae* liquid cultures were incubated at 30°C for 45 min. in YEP medium with or without 2% galactose to induce galactose (*GAL)* genes. Following cross-linking, cell lysis, and DNA shearing, ChIP was performed using an antibody specific for RNA polymerase 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 specific to the *GAL* regions and the products were subsequently analyzed by agarose gel electrophoresis.

Reagent Preparation

Before starting, add 24 ml 100% ethanol to the 6 ml **DNA Wash Buffer** concentrate to obtain the final **DNA Wash Buffer** solution.

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

Protocol

1. In a 1.5 ml microcentrifuge tube, add 5 volumes of **ChIP DNA Binding Buffer** to each volume of sample (5:1). Mix briefly.

   Example 1: Add 250 µl **ChIP DNA Binding Buffer** to 50 µl cell lysate following DNA shearing, reverse cross-linking and Proteinase K digestion in TES (50 mM Tris-Cl, pH 8.0, 10 mM EDTA, 1% SDS) or 0.1M $NaHCO_3$ containing 1% SDS .

   Example 2: Add 600 µl **ChIP DNA Binding Buffer** to 120 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.

   Note: For clean-up of DNA from most enzymatic reactions, add five volumes of ChIP DNA Binding Buffer to each volume of sample (i.e., 5:1).

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

3. Centrifuge at ≥ 10,000 x *g* for 30 seconds. Discard the flow-through.

4. Add 200 µl **Wash Buffer** to the column. Centrifuge at ≥ 10,000 x *g* for 30 seconds. Repeat wash step.

5. Add 6-100 µl **Elution Buffer** directly to the column matrix. Transfer the column to a new 1.5 ml microcentrifuge tube and centrifuge at ≥ 10,000 x *g* for 30 seconds to elute the DNA.

   Ultra-pure DNA is now ready for use for PCR, arrays, DNA quantification, sequencing and other molecular applications.

Ordering Information

Product Description	Catalog No.	Kit Size
ChIP DNA Clean & Concentrator™ supplied w/ uncapped columns	D5201	50 Preps.
ChIP DNA Clean & Concentrator™ supplied w/ capped columns	D5205	50 Preps.

For Individual Sale	Catalog No.	Amount
ChIP DNA Binding Buffer	D5201-1-50	50 ml
DNA Wash Buffer (concentrate)	D4003-2-6	6 ml
DNA Elution Buffer	D3004-4-10	10 ml
Zymo-Spin™ I Columns (uncapped) 	C1003-50 C1003-250	50 columns 250 columns
Zymo-Spin™ IC Columns (capped) 	C1004-50 C1004-250	50 columns 250 columns
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 tubes 500 tubes 1,000 tubes

Popular 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.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns.	**D5001** (spin column) **D5002** (spin column) **D5003** (shallow-well plate) **D5004** (deep-well plate)
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.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns.	**D5005** (spin column) **D5006** (spin column) **D5007** (shallow-well plate) **D5008** (deep-well plate)
EZ DNA Methylation-Direct™ Kit	Features 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.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns.	**D5020** (spin column) **D5021** (spin column) **D5022** (shallow-well plate) **D5023** (deep-well plate)
Methylated DNA Standards			
Universal Methylated DNA Standard	pUC19 plasmid 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	**D5010**
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**
Other…			
ChIP DNA Clean & Concentrator™	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. 50 Preps.	**D5201** (uncapped column) **D5205** (capped column)
Zymo*Taq*™ DNA Polymerase	Zymo*Taq*™ 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. Available either as a single buffer premix or as a polymerase system with components provided separately.	50 Rxns. 200 Rxns. 50 Rxns. 200 Rxns.	**E2001** (system) **E2002** (system) **E2003** (premix) **E2004** (premix)