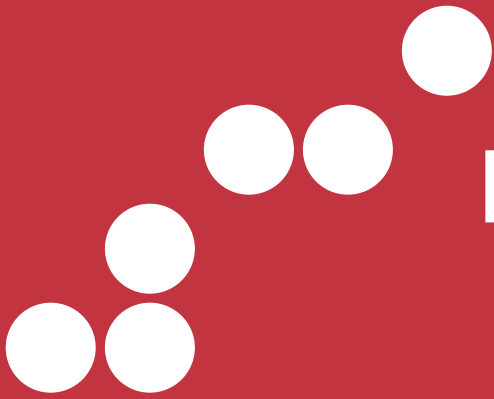


Instruction Manual



MicroChIP DiaPure columns

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Content

Introduction.....	4
Product Contents.....	4
Specifications.....	4
Product Description.....	5
Reagent Preparation.....	6
Protocol.....	7

Introduction

- 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 ($\geq 6 \mu\text{l}$) 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.

Product Contents

Description	Quantity	Storage
ChIP DNA Binding buffer	50 ml	Room Temperature
DNA Wash buffer*	6 ml	Room Temperature
DNA Elution buffer	10 ml	Room Temperature
Spin columns	50 columns	Room Temperature
Collection tubes	50 tubes	Room Temperature
Instruction Manual	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**.

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 crosslinking 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 NaHCO₃ 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** – $\leq 5\%$ Triton X-100, $\leq 5\%$ Tween-20, $\leq 5\%$ Sarkosyl, $\leq 1\%$ SDS.

Note - 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 crosslinking procedure and Proteinase K digestion, the DNA is isolated for analysis.

The **MicroChIP DiaPure columns** provide 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.*

ChIP sample



Ultra-pure DNA is ideal for:

- Library Preparation
- PCR analysis
- Southern blot analysis
- DNA quantification

Figure 1: Two minute MicroChIP DiaPure procedure.

The MicroChIP DiaPure columns employ a single buffer system that allows for efficient DNA adsorption to the matrix of the supplied 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 **Spin column**. There is no need for organic extraction or ethanol precipitation. Instead, Fast-Spin column technology yields ultrapure DNA in just minutes. The DNA purified using the **MicroChIP DiaPure columns** 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 NaHCO₃, 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 **MicroChIP DiaPure columns** 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 crosslinked, treated with Proteinase K, and the DNA purified using the **MicroChIP DiaPure columns**.

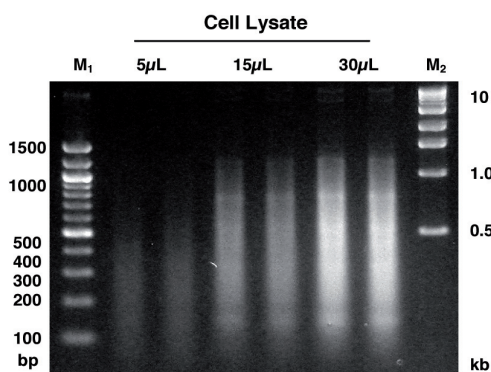


Figure 2: Agarose gel electrophoresis of DNA isolated from cell lysates.

High quality DNA can be efficiently recovered from *Saccharomyces cerevisiae* cell lysates using the MicroChIP DiaPure columns. 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 M1 and M2 are 100 bp and 1 kb ladders, respectively.

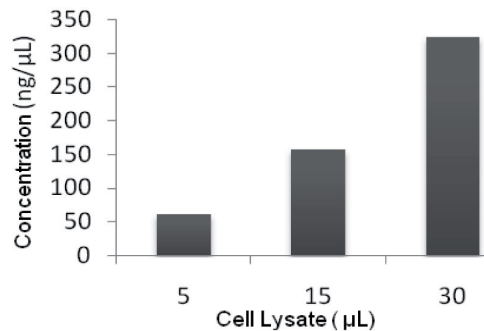


Figure 3: Quantitative recovery of DNA from cell lysates.

The MicroChIP DiaPure columns were 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 spectrophotometry.

The **MicroChIP DiaPure columns** 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.

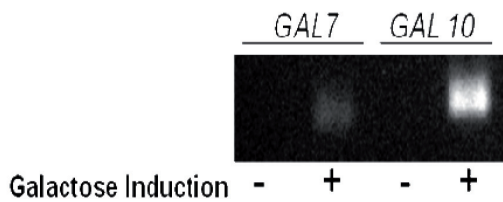


Figure 4: Yeast ChIP PCR Analysis.

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 MicroChIP DiaPure columns. 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.

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 μ l ChIP DNA Binding buffer to 50 μ l 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 NaHCO₃ containing 1% SDS.

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).

Example 2: Add 600 μ l ChIP DNA Binding buffer to 120 μ l 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 mixture to a provided Spin column in a Collection tube.

3. Centrifuge at $\geq 10,000 \times g$ for 30 seconds. Discard the flow-through.

4. Add 200 μ l DNA Wash buffer to the column. Centrifuge at $\geq 10,000 \times g$ for 30 seconds. Repeat wash step.

5. Add 6-100 μ l DNA Elution buffer directly to the column matrix. Transfer the column to a new 1.5 ml microcentrifuge tube and centrifuge at $\geq 10,000 \times g$ for 30 seconds to elute the DNA.

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



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