Chromatin IP DNA Purification Kit

(version A3)

Catalog No. 58002

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Overview

Chromatin Immunoprecipitation (ChIP) is a powerful, well established technique for studying interactions between chromatin-associated proteins and specific regions of the genome. The use of ChIP in combination with genome-wide analysis techniques can yield a tremendous amount of information regarding the distribution of transcription factors and histone modifications. But, these techniques require DNA that has been purified away from the components and contaminants present in an eluted ChIP sample. Active Motif's Chromatin IP DNA Purification Kit enables you to quickly clean up your ChIP DNA samples and get them ready for analysis, without the need for messy, labor intensive and time consuming phenol/chloroform extraction.

The Chromatin IP DNA Purification Kit is designed to be used to purify DNA following chromatin immunoprecipitation. It contains all necessary reagents to purify DNA from 50 ChIP reactions and can be used with material derived from any of the Active Motif line of ChIP-IT® Kits, including our ChIP-IT® Protein G Magnetic Beads (Catalog No. 53014).

product	format	catalog no.
Chromatin IP DNA Purification Kit	50 rxns	58002

Kit Components and Storage

Please store each component at the temperature indicated in the table below.

Reagents	Quantity	Storage / Stability
DNA Purification Binding Buffer	50 ml	Room temperature
DNA Purification Wash Buffer*	50 ml	Room temperature
DNA Purification Elution Buffer	5 ml	Room temperature
3M Sodium Acetate**	500 µl	Room temperature
DNA purification columns	50	Room temperature
DNA column collection tubes	50	Room temperature

Columns and buffers (with the exception of the 3M Sodium Acetate) can be stored at 4ºC, but all components must be warmed to room temperature before use. Some of the salts present in the buffers may precipitate out of solution during storage and will need to be resuspended prior to use.

* DNA Purification Wash Buffer must be reconstituted to a final concentration of 80% ethanol prior to use.

** **Do not** store the 3M Sodium Acetate at 4ºC.

Additional materials required

- Ethanol, 100%
- Microcentrifuge
- 1.5 or 2 ml microcentrifuge tubes

Chromatin IP DNA Purification Kit Experimental Design

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Prior to using the Chromatin IP DNA Purification Kit for the first time, the DNA Purification Wash Buffer must be reconstituted to a final concentration of 80% ethanol using 100% ethanol supplied by the user. This only needs to be performed once, prior to first using the kit. After reconstitution, store the buffer at room temperature. See step A1 in on page 4.

The Chromatin IP DNA Purification Kit is designed for use with a microcentrifuge for sample processing but can also be used with a vacuum manifold. Please consult the manufacturer's instructions for adaptation of the kit for vacuum manifold use.

Compatibility: Once your ChIP experiments are complete, the DNA purification procedure can be started immediately. The entire procedure should take no more than five to ten minutes, depending upon the number of samples to purify. The Chromatin IP DNA Purification Kit is compatible with samples from all Active Motif ChIP-IT Kits, or from any standard chromatin IP kit or procedure, using either mechanical or enzymatic shearing of chromatin, agarose or paramagnetic beads. Before purification, DNA samples should be eluted from the beads, cross-link reversed, and, if the ChIP procedure calls for it, Proteinase K and/or RNase digested.

Binding of DNA to Column: One critical aspect of the procedure is the pH of the sample when it is added to the column. Binding of DNA to the silica matrix of the DNA purification column will only occur at or below pH 7.5. A pH indicator dye is present in the DNA Purification Binding Buffer so that you can easily monitor that your samples are at the correct pH. To ensure successful purification of your ChIP DNA, sodium acetate (pH 5.4) is supplied in case the pH of your sample needs to be adjusted lower.

Washing: DNA bound to the column is extensively washed after binding.

Elution: After washing, the DNA is eluted using the included DNA Purification Elution Buffer. Elution can be carried out using highly pure water (pH 7) if desired.

Purity: Use of the Chromatin IP DNA Purification Kit results in recovery of purified DNA of high quality suitable for a number of downstream analysis techniques, including PCR (endpoint or quantitative), Southern blotting, microarray, standard or Next-Gen sequencing or cloning.

Recovery: DNA fragments below 50 base pairs in length are not recovered efficiently.

Yield: Recovery of DNA will be 85-100%. Depending upon the number of cell equivalents of chromatin used in each ChIP reaction, this typically will be from 100 ng to 1 µg. DNA can be successfully recovered from ChIP experiments starting with as few as 10,000 cells.

A. Purification of DNA from Chromatin IP Samples

1. **ADD ETHANOL BEFORE THE FIRST USE ONLY!** The final concentration of ethanol in the DNA Purification Wash Buffer must be 80%. Add 40 ml of fresh 100% ethanol to the DNA Purification Wash Buffer bottle. Invert repeatedly. The buffer can be stored at room temperature after the addition of ethanol.
2. Your eluted ChIP samples should be in an Eppendorf tube. To each sample, add 5 volumes DNA Purification Binding Buffer for every volume of DNA sample. For example, if your ChIP sample is 100 µl, add 500 µl Binding Buffer.
3. Add 5 µl 3M Sodium Acetate and mix. Check that the color of the DNA sample / DNA Purification Binding Buffer mixture is bright yellow, not light orange or violet. If the color is light orange or violet, this indicates that its pH is too high. Add more 3M Sodium Acetate 5 µl at a time, mixing after addition, until the color is bright yellow (see Figure 1). As the elution buffers used in many ChIP protocols (including those in the ChIP-IT Kits) have a basic pH, **this step is crucial to the successful binding and purification of your ChIP DNA sample.**

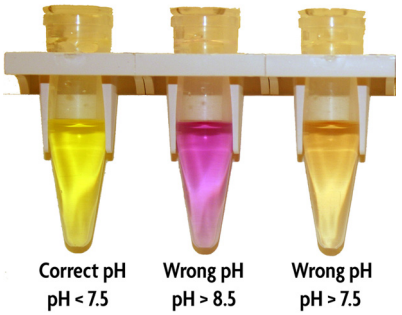

Figure 1: Solution color as a function of pH.
The DNA Purification Binding Buffer has a pH indicator dye, so that the pH of the solution can be easily determined. Only apply the sample to the column when the solution is bright yellow (left), indicating a pH under 7.5. DNA will not bind to the column if the pH is higher than 7.5.

Note: A full-color PDF of this manual can be downloaded from the Active Motif website, which will enable you to see this figure in color.

4. For each sample, place a DNA purification column in the collection tube and add each sample to its own column. Close the cap on each column, place them with the collection tubes in a microcentrifuge and spin them at 14,000 rpm for 1 minute. Alternatively, a compatible vacuum manifold may be used for sample processing.
5. Remove the column from the collection tube, then remove and discard the flow through from the collection tube. Return the column to the collection tube.
6. Add 750 µl of Wash Buffer to the column and cap the column.
7. Spin at 14,000 rpm for 1 minute in a microcentrifuge.
8. Remove the column from the collection tube, then remove and discard the flow through from the collection tube. Return the column to the collection tube.
9. With the column cap open, spin at 14,000 rpm for 2 minutes in a microcentrifuge to remove any residual Wash Buffer from the column.

10. Transfer the column to a clean Eppendorf tube.
11. Add 50 µl of DNA Purification Elution Buffer (or purified water with a pH equal to or greater than 7) to the center of the column matrix and wait for 1 minute.

    Note: The amount of Elution Buffer used can be reduced to less than 50 µl in order to attain a more concentrated DNA sample, if required. Reducing the elution volume will result in an overall reduction in the recovery efficiency of the purification. For more information, please see Appendix, Section B, Figure 5.

12. Spin at 14,000 rpm for 1 minute in a microcentrifuge.
13. Discard column. The DNA eluted into the Eppendorf tube is purified and ready to use in your application.

Appendix

Section A. Supplementary Chromatin IP Data

The Chromatin IP DNA Purification Kit is designed for purification of DNA after performing a ChIP experiment. Below are examples of quantitative PCR (qPCR) data from DNA purified using the Chromatin IP DNA Purification Kit after ChIP.

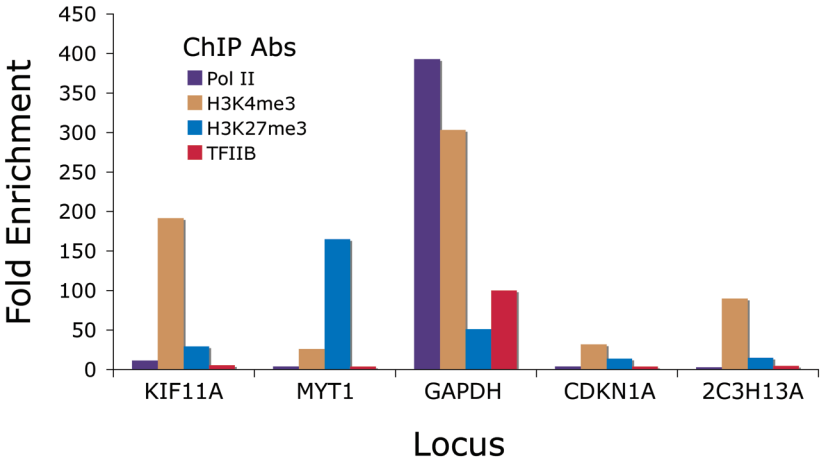

Figure 2: Quantitative PCR performed on ChIP DNA purified with the Chromatin IP DNA Purification Kit.

Quantitative PCR was performed using DNA purified with the Chromatin IP DNA Purification Kit after ChIP using the indicated antibodies (ChIP Abs). Chromatin IP experiments were performed using the ChIP-IT® Express Kit (Catalog No. 53008) and Ready-to-ChIP HeLa Chromatin (Catalog No. 53015, 7.5 x 10^5 cell equivalents per ChIP). Quantitative PCR was carried out using primers specific for the indicated gene (Locus) and normalized data represent fold enrichment over ChIP experiments carried out with control IgG.

Section B. DNA Recovery

The Chromatin IP DNA Purification Kit enables recovery of DNA fragments as small as 50 base pairs (Figure 3). As nucleosomal DNA is on the order of 150 base pairs, all of your chromatin IP DNA should be recovered efficiently.

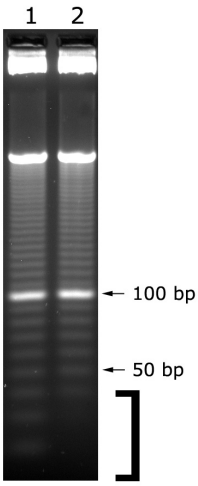

Figure 3: DNA recovery as a function of fragment size.

The Chromatin IP DNA Purification Kit enables efficient recovery of DNA fragments as small as 50 base pairs. DNA molecular weight markers (10 base pair ladder) were purified with the Chromatin IP DNA Purification Kit and run on a 5% agarose gel and stained with ethidium bromide to visualize the recovered DNA fragments.

Lane 1: DNA not purified.
Lane 2: DNA after purification.

As the figure illustrates, DNA larger than 50 base pairs is represented equally in the purified and non-purified DNA samples. DNA fragments smaller than 50 base pairs (bracket) are not efficiently purified, and thus are not represented in the purified DNA sample (Lane 2, bracket).

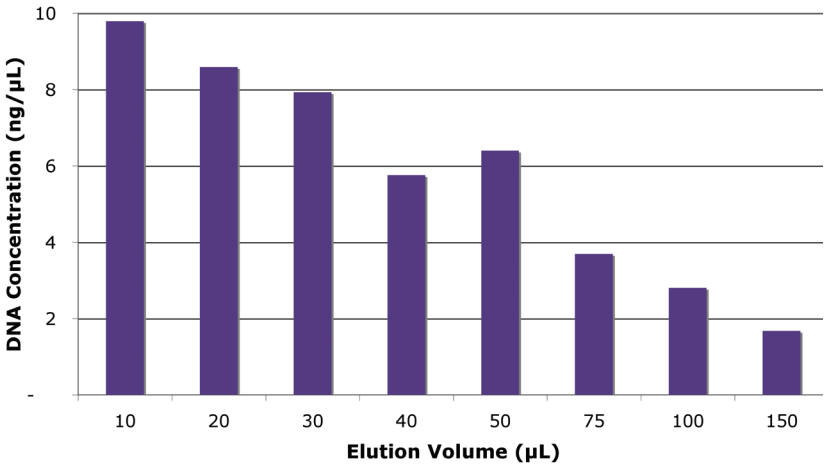

Figure 4: Concentration of DNA recovered as a function of elution volume.

Human genomic DNA was purified using the Chromatin IP DNA Purification Kit and eluted using the indicated volume of Elution Buffer and the concentration (ng/µl) of the recovered DNA was determined.

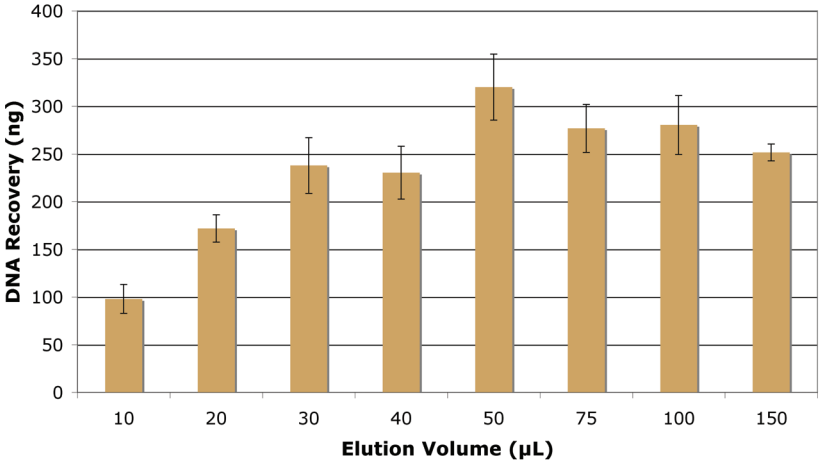

Figure 5: Yield of DNA recovered as a function of elution volume.

Human genomic DNA was purified using the Chromatin IP DNA Purification Kit and eluted using the indicated volume of Elution Buffer and the quantity (ng) of the recovered DNA was determined.

Section C. Troubleshooting Guide

Problem/question	Recommendation
No amplification by PCR of ChIP DNA	If you are experiencing problems with downstream analysis of ChIP DNA, it is possible that the purification process was compromised and your DNA samples were not recovered. This could be due to one of several factors. 1. **The pH of the Binding Buffer was too high.** Refer to Figure 1 on page 4 to make sure that the pH of your sample is below 7.5 to ensure proper binding of ChIP DNA to the column. Your sample should be bright yellow (not orange, peach or purple). Add 3M Sodium Acetate in aliquots of 5 µl until the pH reaches the proper point and the sample is bright yellow. (A full-color PDF of this manual can be downloaded from the Active Motif website, which will enable you to see what is meant by "bright yellow".) 2. **No ethanol in the DNA Purification Wash Buffer.** Make sure that ethanol has been added to the DNA Purification Wash Buffer prior to use. See step A1 on page 4. 3. **Residual DNA Purification Wash Buffer in the column prior to elution.** Make certain to spin the column one extra time after washing to remove any residual Wash Buffer, as it will interfere with elution of the DNA from the column. Open the cap of the column to enable proper removal of residual Wash Buffer. 4. **Salt in the Elution Buffer.** DNA will not be eluted from the silica matrix of the DNA purification column if the buffer used for elution contains high salt. Use the included DNA Purification Elution Buffer or purified water (pH 7) for elution.

Section D. Frequently Asked Questions (FAQ)

Can the Chromatin IP DNA Purification Kit be...

used after 5-hydroxymethylcytidine MeDIP? Yes.

used after 5-methycytidine MeDIP? Yes.

Note: The MeDIP procedure using the 5-Methylcytidine antibody (Catalog No. 39649) requires the DNA to be made single stranded. Single-stranded DNA does not bind as efficiently to the silica matrix of the column so recovery may not be 100%, but our kit has been shown to work as well as the leading competitor's kit.

used with other Active Motif kits, such as the hMeDIP Kit and the MethylCollector™ family? Yes.

used to purify DNA samples from other manufacturer's ChIP kits? Yes.

used to purify DNA samples from non-kit based ChIP protocols? Yes.

used with a vacuum manifold? Yes.

used to purify RNA? Not recommended. The buffers are not guaranteed to be RNase-free.

used to purify PCR products? Yes.

used to clean up restriction enzyme reactions? Yes.

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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