

Enzyme-Based Chromatin Digestion vs. Sonication-Based Chromatin Fragmentation:

A Chromatin IP Methods Comparison from Cell Signaling Technology®

The chromatin immunoprecipitation (ChIP) assay is a powerful technique used for probing protein-DNA interactions within the natural context of the cell. This assay is used to analyze histone modifications and binding of transcription factors, DNA replication factors and DNA repair proteins across the genome. When performing the ChIP assay, cells are first fixed with formaldehyde, which preserves the protein-DNA interactions occurring in the cell. Cells are then lysed and chromatin is harvested and fragmented using either sonication or enzymatic digestion. The fragmented chromatin is subjected to immunoprecipitation using antibodies specific to a particular protein or histone modification. The immunoprecipitated DNA is purified and the enrichment of particular DNA sequences or regions of the genome can be determined by a number of techniques.

The purpose of this study is to compare Cell Signaling Technology's SimpleChIP® Enzymatic Chromatin IP Kits to a competitor's sonication-based ChIP kit to determine the downstream effects of micrococcal nuclease enzyme-based chromatin digestion versus sonication-based chromatin fragmentation. While sonication has been the traditional method used for fragmenting chromatin, many problems can occur due to variability in sonication power and the emulsification of the chromatin sample during sonication. While under-sonication can lead to incomplete chromatin fragmentation, over-sonication or emulsification of the sample can result in the loss of antibody epitopes due to protein denaturation and degradation. We found that enzymatic digestion is much milder than sonication and leads to a dramatic increase in immunoprecipitation efficiencies in the ChIP assay.

We prepared chromatin from HCT116 cells and performed chromatin immunoprecipitations using both kits to analyze histone modifications and TCF4, β -catenin and RNA polymerase (Rpb1) occupancy at several genetic loci. We found that Tri-methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751 and Acetyl-Histone H3 (Lys9/Lys14) Antibody #9677 gave 2- to 3-fold higher enrichment of active genes (GAPDH and c-MYC) when using the SimpleChIP® Enzymatic ChIP Kit (see Figure 1). Enrichment of the inactive MYT-1 gene was minimal with

both kits. Furthermore, the enrichment of active genes using the Rpb1 CTD (4H8) Mouse mAb #2629 was 11-fold and 13-fold higher for GAPDH and c-MYC when using the SimpleChIP® Enzymatic ChIP Kit (Figure 2A). Strikingly, the difference between the kits was most evident when analyzing TCF4 and β -catenin binding. Enrichment of CAMK2D and c-MYC using TCF4 (C48H11) Rabbit mAb #2569 was 4.5-fold and 12.5-fold higher, while enrichment using β -Catenin Antibody #9562 was 34-fold and 4-fold higher when using the SimpleChIP® Enzymatic ChIP Kit (Figure 2B). The enrichment of both loci with the β -catenin antibody was equivalent to background when using the sonication-based ChIP kit.

Our study shows that Cell Signaling Technology's SimpleChIP® Enzymatic Chromatin IP Kit performs much better than the competitor's sonication-based ChIP kit and that micrococcal nuclease digestion produces chromatin fragments that are more conducive to immunoprecipitation. Importantly, this results in increased enrichment of target loci while maintaining low levels of non-specific background signal. This is especially evident when analyzing the binding of transcription factors and cofactors, suggesting that enzymatic digestion is much milder than sonication and better preserves the integrity of the chromatin and antibody epitopes.

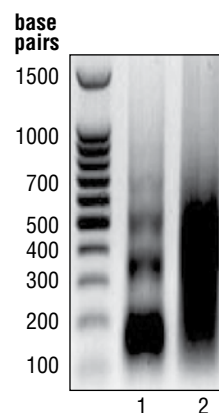


Figure 1.

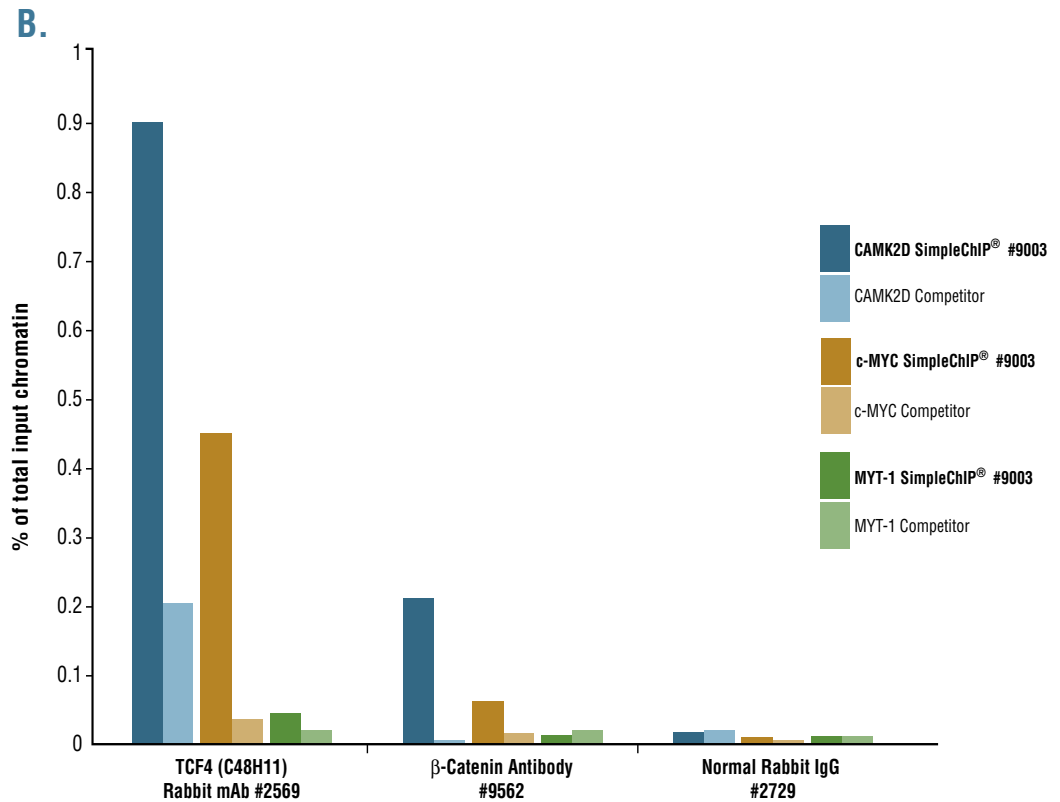
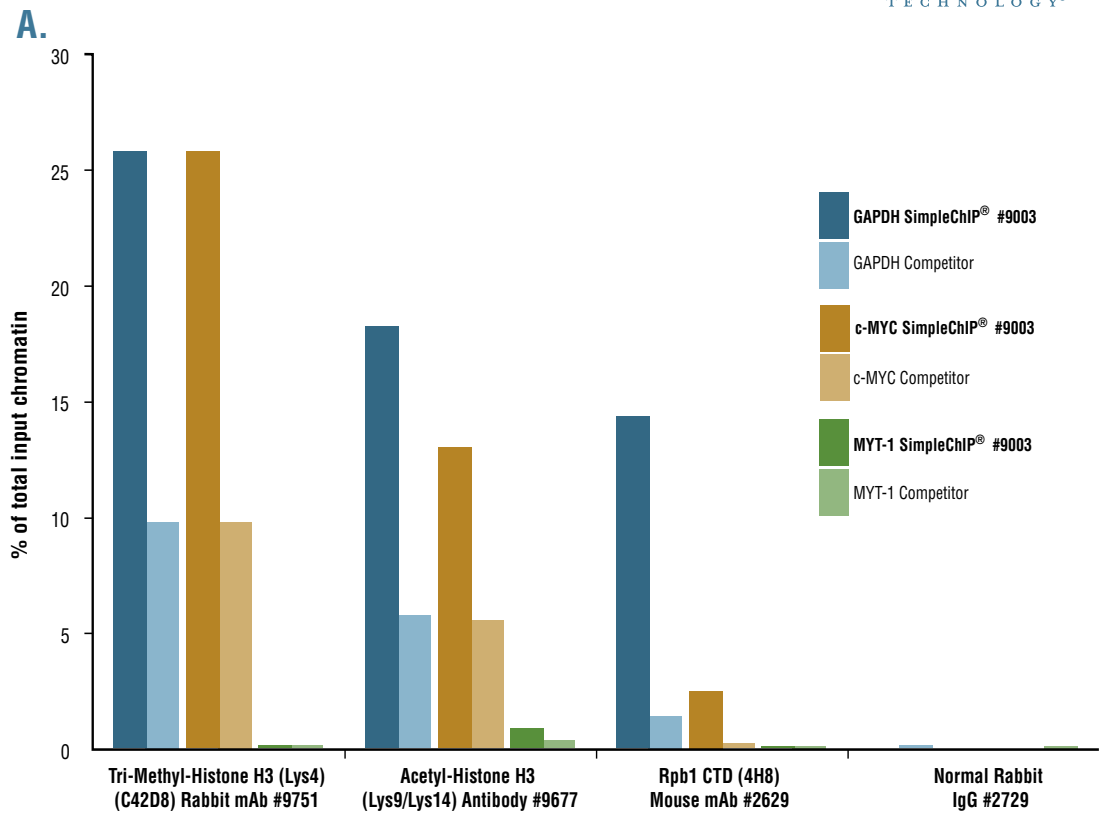
Enzyme-based and sonication-based ChIP kits produce chromatin fragments of a similar size. Chromatin was prepared from 4×10^7 HCT116 human colorectal carcinoma cells according to the protocols included with the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 or a competitor's sonication-based ChIP kit. DNA was purified from each chromatin sample and DNA fragment size was determined by electrophoresis on a 1% agarose gel. Both enzymatic digestion with the SimpleChIP® Kit (lane 1) and sonication with the competitor's kit (lane 2) produced chromatin fragments ranging from 150 to 700 bp, corresponding to one to five nucleosomes in length. The experiment was repeated at least three times with similar results.

Figure 2

SimpleChIP® digested chromatin is more conducive to immunoprecipitation than sonicated chromatin. Chromatin immunoprecipitations were performed with 10 µg of cross-linked HCT116 chromatin and the indicated antibodies, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 or a competitor's sonication-based ChIP Kit. The enriched DNA was quantified by qPCR. The amount of immunoprecipitated DNA in each sample is presented as a percent of the total input chromatin. The experiment was repeated at least three times with similar results.

A. PCR primers are specific for the transcriptionally active GAPDH and c-MYC genes and the inactive MYT-1 gene.

B. PCR primers are specific for known TCF4 binding sites in the CAMK2D and c-MYC genes, and a region of the MYT-1 gene that does not contain a TCF4 binding site.



New ChIP Kits

SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) #9002

SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003

New Companion Products

6-Tube Magnetic Separation Rack #7017

ChIP-Grade Protein G Magnetic Beads #9006

ChIP-Grade Protein G Agarose Beads #9007

Please visit www.cellsignal.com for more information and for a complete list of our ChIP-validated antibodies.

For questions regarding ChIP kits and ChIP-validated antibodies, please contact Christopher Fry, Ph.D. or Curtis Desilets at CST_ChIP@cellsignal.com.