The Use of Highly Validated Recombinant Rabbit Monoclonal Antibodies to Analyze Histone Modifications and Transcription Factor Binding by ChIP-seq

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ABSTRACT

Research in the field of epigenetics has grown at a rapid pace since the discovery of the first histone acetyltransferase enzymes 20 years ago. Since then, significant advances have been made in our understanding of the basic epigenetic mechanisms regulating gene expression and genomic stability, and the impact of epigenetic deregulation on cancer, inflammation, metabolism, and neurological diseases. Much of our knowledge of the these mechanisms comes from the utilization of antibodies to probe the localization of transcription factors, chromatin regulators, and histone modifications in different cell and tissue types, and across the genomes of a multitude of organisms using ChIP-qPCR and ChIP-seq. Like most assays, the robustness and reliability of ChIP-qPCR and ChIP-seq data is highly dependent on the antibodies used. Currently, polyclonal antibodies are the standard reagent used by many labs and consortia, including ENCODE and NIH Roadmap Epigenomics projects. However, polyclonal antibodies are non-renewable reagents that can show considerable variability in performance between lots, resulting in the need to re-validate each new lot of antibody. Monoclonal antibodies, which are renewable and provide much more consistent performance between lots, provide a valuable alternative to polyclonal antibodies. We will demonstrate how the utilization of rabbit monoclonal antibody technology, combined with thorough antibody validation can lead to the generation of high quality recombinant rabbit monoclonal antibodies that show exquisite specificity, sensitivity, and reproducibility, and provide superior performance in ChIP-qPCR and ChIP-seq assays.

INTRODUCTION

Like most assays, the robustness and reliability of ChIP-seq data is highly dependent on the antibody used. At Cell Signaling Technology, we have developed our own proprietary recombinant rabbit monoclonal antibody technology that we combine with thorough antibody characterization and validation to develop antibodies that show exquisite specificity and sensitivity across multiple applications (1). Our recombinant rabbit monoclonal antibodies provide many advantages over polyclonal antibodies, often showing higher specificity, less lot-to-lot variability, and equal or superior performance in multiple applications (2). In fact, Busby et al. 2016 describe a number of CST rabbit mAbs that perform as well as or better than polyclonal antibodies in ChIP-seq (3).

Chromatin preparation and fragmentation is also critical to a successful ChIP experiment. At Cell Signaling Technology, we have developed two methods for chromatin fragmentation that are highly compatible with transcription factor and cofactor ChIP-qPCR and ChIP-seq. First, our SimpleChIP® Plus Enzymatic Chromatin IP Kit #9005 utilizes micrococcal nuclease (MNase) to digest chromatin at low temperature in a low detergent buffer, providing a mild fragmentation method that maintains the structure and integrity of the chromatin and bound proteins. Second, we have now developed a SimpleChIP® Plus Sonication protocol that also maintains chromatin integrity during sonication. As demonstrated, both of these methods provide robust ChIP-seq results when combined with our recombinant rabbit mAbs against histones, transcription factors and cofactors.

SUMMARY

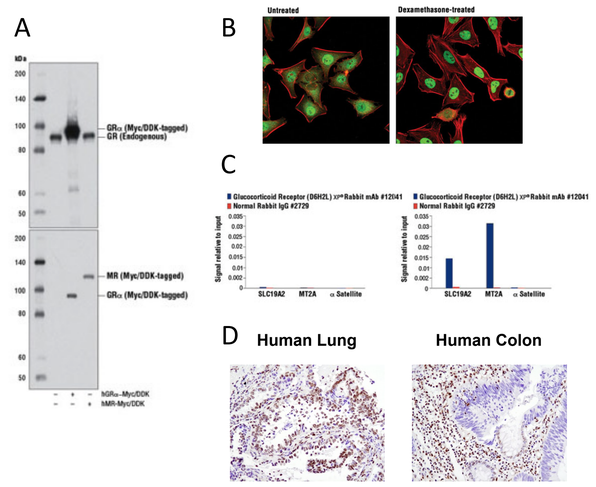
- CST provides recombinant rabbit mAbs that are thoroughly validated for specificity, sensitivity, and reproducibility across multiple applications using biologically relevant cell and tissue model systems.
- Antibodies are optimized for every application and come with recommended dilutions and protocols.
- Our data show that recombinant rabbit mAbs provide better lot-to-lot consistency, specificity, and sensitivity than polyclonal antibodies across multiple applications.
- CST has a large portfolio of over 260 ChIP-qPCR validated antibodies and over 40 ChIP-seq validated antibodies.
- CST's SimpleChIP® Plus Enzymatic Chromatin IP kits and SimpleChIP® Plus Sonication protocol provide superior performance than leading competitor kits for transcription factor and cofactor ChIP-seq.
- For a complete list of ChIP products, please visit our website at www.cellsignal.com/epigenetics

REFERENCES

1. Cheung W.C. et al. (2012) *Nat Biotechnol* 30, 447-452.
2. Peach S.E. et al (2012) *Mol Cell Proteomics* 11, 128-37.
3. Busby M. et al. (2016) *Epigenetics & Chromatin* 9, 49-65.

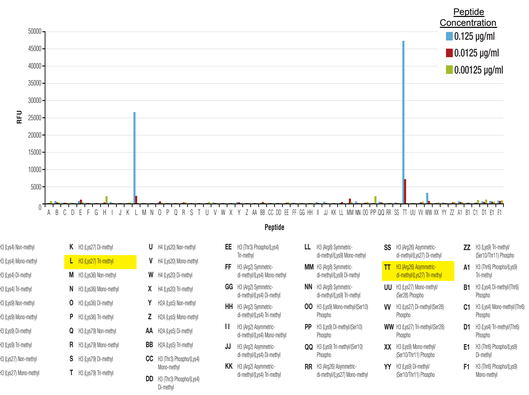
Antibody Validation

Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb #12041

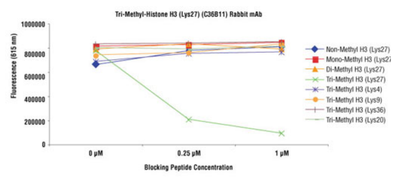


Validation of Glucocorticoid Receptor (GR) rabbit mAb using overexpression and cell treatments. (A) WB analysis of GR on 293T cell extracts, mock transfected or transfected with constructs expressing Myc-DDK-tagged full-length human GR-α or full-length human Mineralocorticoid Receptor (MR). In the upper panel, extracts are probed with Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb #12041 showing increased signal with GR-α transfection, but no detectable increase with MR transfection. In the lower panel, extracts are probed with DYKDDDDK Tag Antibody #2368 showing appropriate expression of each tagged protein. **(B)** IF with the GR antibody on HeLa cells, either untreated or dexamethasone-treated. Appropriate nuclear translocation is observed when cells are treated with dexamethasone. **(C)** ChIP-qPCR was performed on A549 cells either untreated or treated with dexamethasone using SimpleChIP® Plus Enzymatic Chromatin IP Kit #9005. Treatment with dexamethasone induces recruitment of GR to target genes. **(D)** IHC analysis of paraffin-embedded human lung and human colon carcinoma showing appropriate staining of GR in each tissue.

Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733



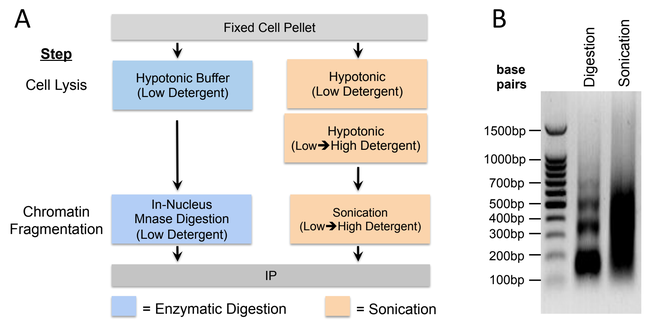
Histone peptide array assay showing Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733 is highly specific for tri-methyl histone H3 lysine 27 and is not affected by methylation of the neighboring arginine 26 residue.



Histone peptide competition ELISA showing Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733 is highly specific for tri-methyl histone H3 lysine 27. The graph depicts the binding of antibody to pre-coated tri-methyl-histone H3 (Lys27) peptide in the presence of increasing concentrations of various competitor peptides. As shown, only the tri-methyl histone H3 (Lys27) peptide competed away binding of the antibody.

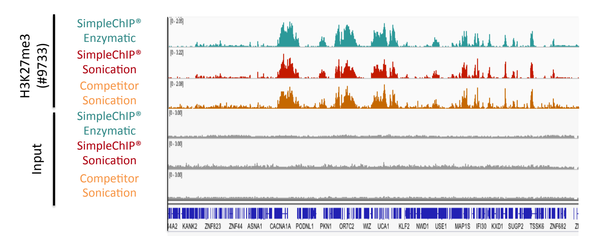
ChIP-seq Methods Enzymatic vs. Sonication

Key Differences in Chromatin Fragmentation



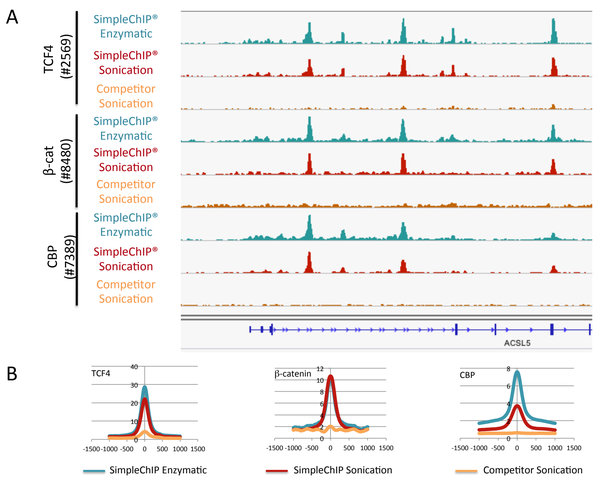
Chromatin can be fragmented prior to IP by two different methods: enzymatic digestion or sonication. (A) Enzymatic digestion (blue) involves the use of Micrococcal nuclease (MNase), an enzyme which cuts naked double-stranded DNA between nucleosomes. This digestion is done in low detergent buffer at 37°C, and provides a mild method for fragmentation of cross-linked chromatin. Incomplete digestion allows for generation of chromatin fragments from 150 to 750 bp in length, which is ideal for chromatin IP. Sonication (orange) involves the physical shearing of chromatin. Multiple rounds of sonication using a probe sonicator, water bath sonicator, or ultrasonicator are used to shear chromatin. A relatively high detergent buffer is typically required to generate proper sized fragments, and a large amount of heat is generated during sonication, both of which can denature chromatin and antibody epitopes. **(B)** Agarose gel showing fragment size of DNA purified from digested or sonicated chromatin.

Histones are Resistant to Harsh Sonication Conditions



ChIP-seq was performed using cross-linked chromatin from HeLa cells and Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733, using either the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005 (blue), the SimpleChIP® Plus sonication ChIP protocol (red), or a competitor sonication ChIP kit and protocol (orange). Chromatin fragmentation by sonication was performed using a probe sonicator. DNA libraries were prepared from 50 ng of enriched ChIP DNA and sequenced on the Illumina® NextSeq®. As shown in the localized ChIP-seq tracks, tri-methyl-histone H3 lysine 27 ChIP is resistant to the harsh conditions associated with sonication-based chromatin fragmentation.

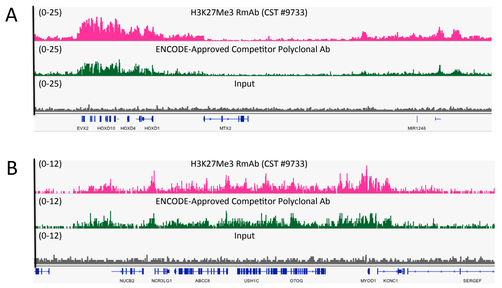
Transcription Factors and Cofactors are Sensitive to Harsh Sonication Conditions



ChIP-seq was performed using cross-linked chromatin from HCT116 cells and CST rabbit mAbs against TCF4 (#2569), β-catenin (#8480) or CBP (#7389), as described above. As shown in the localized ChIP-seq track for chromatin surrounding the ACSL5 gene **(A)** and the metagene analysis depicting the averaged genome-wide enrichment for each antibody **(B)**, TCF4, β-catenin, and CBP ChIP is highly sensitive to the high detergent conditions associated with the competitor's sonication kit, while the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005 and the SimpleChIP® Plus sonication protocols work well for all three targets.

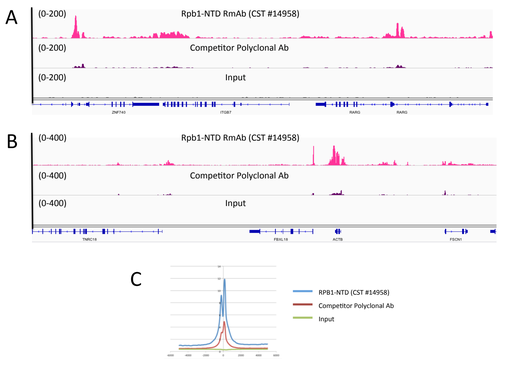
Recombinant Rabbit mAbs for ChIP-seq

Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733



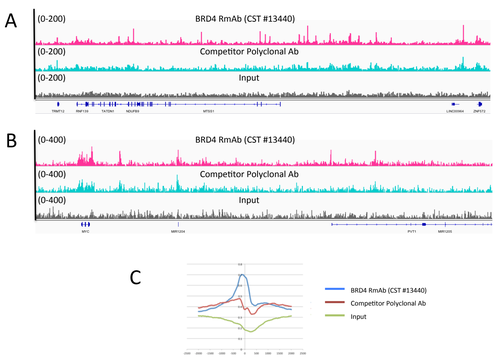
The Recombinant Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733 shows a higher signal to noise ratio than an ENCODE-approved competitor polyclonal antibody. ChIP was performed with cross-linked chromatin from NCCIT cells and either 0.59 µg of H3K27Me3 RmAb #9733 or the recommended 2 µg of the competitor antibody, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA libraries were prepared from 50 ng of enriched ChIP DNA and sequenced on the Illumina® NextSeq®. Localized ChIP-seq tracks are shown for chromatin surrounding the HOXD gene cluster **(A)** and the MYOD gene **(B)**.

Rpb1 NTD (D8L4Y) Rabbit mAb #14958



The Recombinant Rpb1 NTD (D8L4Y) Rabbit mAb #14958 shows a higher signal to noise ratio than a leading competitor polyclonal antibody. ChIP was performed with cross-linked chromatin from HeLa cells and either 2 µg of Rpb1 RmAb #14958 or 2 µg of the competitor antibody, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA libraries were prepared from 5 ng of enriched ChIP DNA and sequenced on the Illumina® NextSeq®. Localized ChIP-seq tracks are shown for chromatin surrounding the ZNF740 gene **(A)** and the ACTB gene **(B)**. **(C)** Metagene analysis shows averaged genome wide signal to noise ratio for enrichment of Rpb1 at transcription start sites.

BRD4 (E2A7X) Rabbit mAb #13440



The Recombinant BRD4 (E2A7X) Rabbit mAb #13440 shows a higher signal to noise ratio than a leading competitor polyclonal antibody. ChIP was performed with cross-linked chromatin from MV-4-11 cells and either 7 µg of BRD4 RmAb #13440 or 7 µg of the competitor antibody, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA libraries were prepared from 5 ng of enriched ChIP DNA and sequenced on the Illumina® NextSeq®. Localized ChIP-seq tracks are shown for chromatin surrounding the MTSS1 gene **(A)** and the MYC gene **(B)**. **(C)** Metagene analysis shows averaged genome wide signal to noise ratio for enrichment of BRD4 at transcription start sites.

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