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-modifying enzymes 25 years ago. Since then, significant advances have been made in our understanding of the mechanisms underlying histone modifications and their roles in regulating gene expression and genomic stability, and the impact of epigenetic deregulation on cancer, inflammation, metabolism, and neurological diseases. Much of our knowledge of 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-reproducible 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 more reproducible, provide a consistent, reliable, and 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 through antibody validation led 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 with consistently superior 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. (2) 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 Kits #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 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 CST 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 CST products, please visit our website at www.cellsignal.com/epiagnostics

REFERENCES

