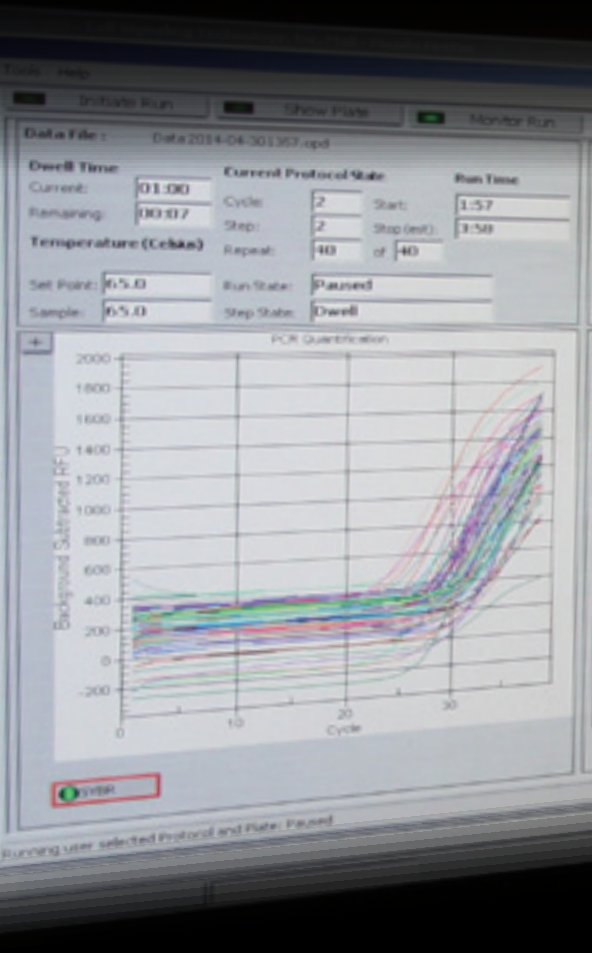
APPLICATION FOCUS

SimpleChIP®

Enzymatic Chromatin IP Kits and ChIP-validated Antibodies

Cell Signaling TECHNOLOGY®

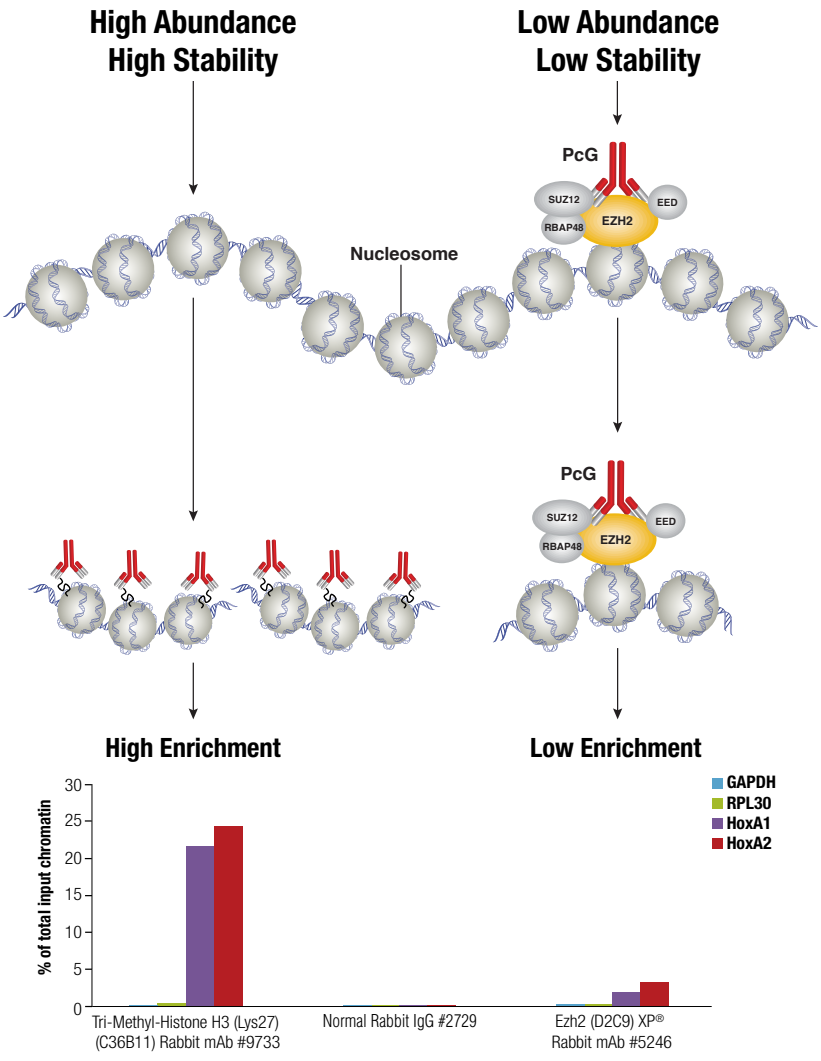
Optimized Reagents Matter



The success or failure of a ChIP experiment is highly dependent on the integrity of the chromatin, the quality of the epitope, and the specificity of the antibody. Just as important is the inclusion of a control antibody that binds at the locus of interest and allows the investigator to confidently assess results. These components must be optimized to work together, especially when the target interaction is a low abundance, low stability event.

High abundance, very stable protein-DNA interactions like those between histones and DNA, occur frequently enough that they may still be detected even if the integrity of the DNA or protein epitopes has been compromised, or if the signal to noise ratio of the antibody is low.

Low abundance, less stable interactions such as the binding of polycomb group proteins (e.g., Ezh2) to specific genes, may fall under the detection threshold if the protocol fails to safeguard the integrity of the protein and the DNA, or if it relies on an antibody that is not highly specific to the target of interest.



CST Technical Support

At CST, providing exceptional customer service and technical support is a top priority. Our scientists work at the bench daily to produce and validate our antibodies, so they have hands-on experience and in-depth knowledge of each antibody's performance. In the process, these same scientists generate valuable reference information that they use to answer your questions and help troubleshoot your experiment by phone or email.

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❶ Chromatin Integrity | Enzymatic Digestion versus Sonication

Sonication uses acoustic energy to mechanically shear the chromatin. While effective, sonication is difficult to control and requires exposing the chromatin to harsh, denaturing conditions (i.e., high heat and detergent) that can damage both antibody epitopes and the genomic DNA.

Enzymatic digestion, on the other hand, uses micrococcal nuclease to gently fragment the chromatin. This approach is simple to control and doesn't require harsh, denaturing conditions. Therefore, enzymatic digestion protects the integrity of the chromatin and antibody epitopes, resulting in a higher quality chromatin preparation that is more conducive to immunoprecipitation.

ChIP was performed using the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005 and a competitor's sonication-based ChIP kit. Chromatin was first prepared using either enzymatic digestion (according to the SimpleChIP® kit instructions) or sonication (according to the competitor's kit instructions). Ten micrograms of chromatin was subjected to immunoprecipitation with the indicated panel of antibodies using immunoprecipitation reagents from either the SimpleChIP® or the competitor's kit. The immunoprecipitated DNA was quantified by qPCR and is presented as a percent of the total input chromatin.

Enzyme digested chromatin showed more robust enrichment of target DNA loci than sonicated chromatin, using either the competitor's IP kit or the SimpleChIP® IP kit. This was especially apparent when less stable interactions, such as the binding of polycomb group proteins (Ezh2 [D] or SUZ12 [E]) to specific genes were assayed.

GAPDH
Digested Chromatin
Sonicated Chromatin

HoxA1
Digested Chromatin
Sonicated Chromatin

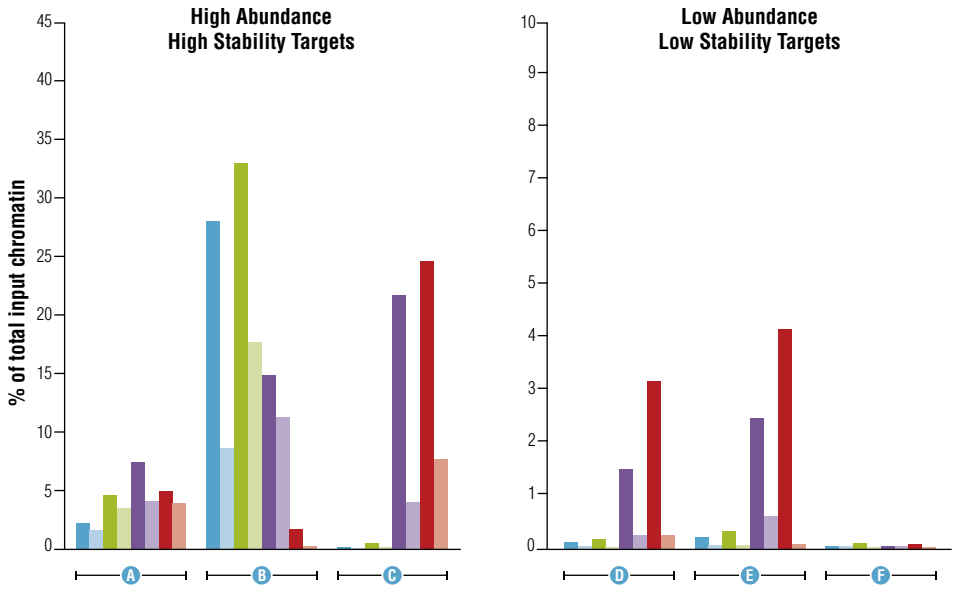
RPL30
Digested Chromatin
Sonicated Chromatin

HoxA2
Digested Chromatin
Sonicated Chromatin

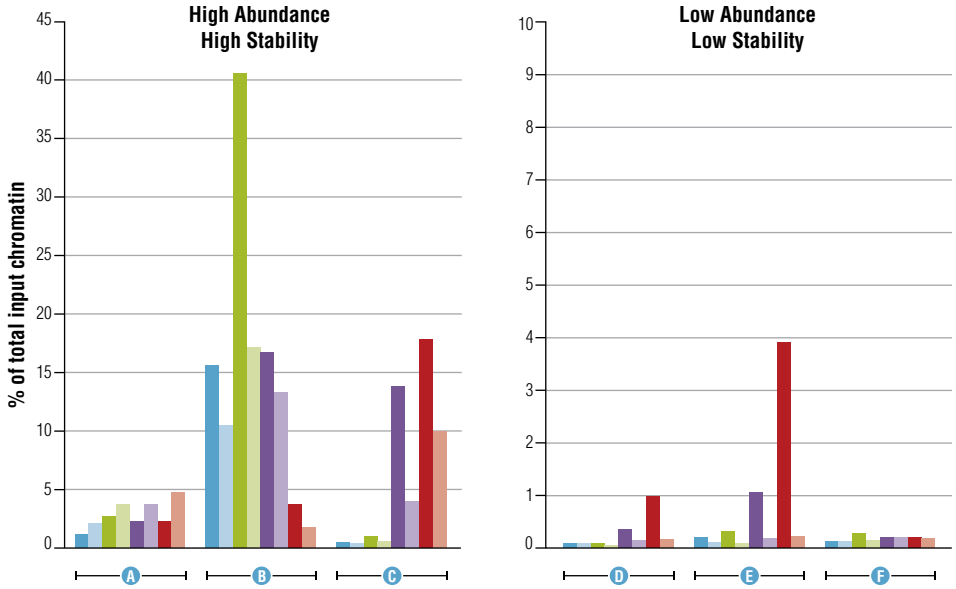
- A Histone H3 (D2B12) XP® Rabbit mAb (ChIP Formulated) #4620
- B Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751
- C Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733
- D Ezh2 (D2C9) XP® Rabbit mAb #5246
- E SUZ12 (D39F6) XP® Rabbit mAb #3737
- F Normal Rabbit IgG #2729

Target DNA loci are immunoprecipitated better from enzyme digested chromatin than sonicated chromatin.

SimpleChIP® IP Protocol



Competitor IP Protocol



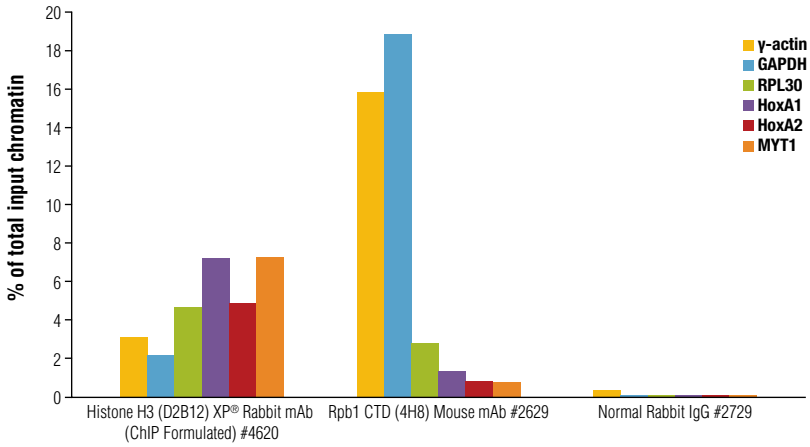
❷ Assay Reliability | Histone H3 versus Rpb1

Rpb1 (largest subunit of RNA pol II) is commonly supplied as a positive control antibody in competitor ChIP kits, but it only binds at active loci, so it may not be an appropriate choice if you are looking at protein-DNA interactions that occur at inactive loci.

SimpleChIP® kits utilize Histone H3 (D2B12) XP® Rabbit mAb (ChIP Formulated) #4620. This antibody detects total H3 protein, which is present at all DNA sequences in the genome. Thus, it provides a reliable positive control for your ChIP experiments, independent of the locus under examination.

Histone H3 showed significant enrichment of the target at every locus tested, while Rpb1 showed good enrichment only at highly active loci (i.e., γ-actin and GAPDH), and limited enrichment at less active loci (i.e., HoxA1, HoxA2).

H3 is a more reliable control than Rpb1



ChIP was performed with 10 µg of cross-linked chromatin and the indicated antibodies. 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.

❸ Antibody Specificity | CST ChIP-Validated Antibodies

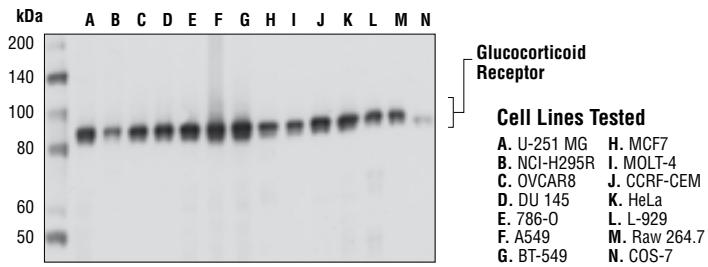
Antibodies that non-specifically bind unintended targets increase the background noise, making it more difficult to detect low abundance interactions.

CST offers antibodies that have been validated to work in ChIP applications, using the same rigorous standards we apply to all our antibodies.

Please visit **www.cellsignal.com/chipab** for a full list of ChIP validated antibodies.

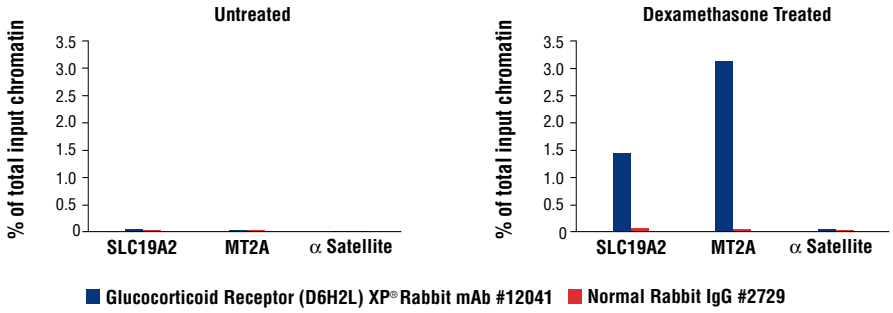
Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb showed efficient target enrichment only when the cells were treated with dexamethasone, indicating the antibody is highly specific for the target of interest.

Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb #12041



Western blot analysis of extracts from various cell lines using Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb. (upper)

A549 cells were cultured in media with 5% charcoal-stripped FBS for 3 d and then either untreated (left panel) or dexamethasone-treated (100 nM, 1 hr; right panel). Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 cells and 10 µl of Glucocorticoid Receptor (D6H2L) Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human SLC19A2 Promoter Primers #7681, human MT2A promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as percent of the total input chromatin. (lower)



SimpleChIP® Plus

Enzymatic Chromatin IP Kits and ChIP-validated Antibodies

SimpleChIP® Plus Chromatin IP Kits from CST detect endogenous protein-DNA interactions in cultured cells and tissue samples.

These kits contain all reagents necessary to perform enzymatic digestion-based chromatin immunoprecipitation (ChIP) experiments quickly and easily, as well as positive and negative controls that allow you to be confident in your results. These kits are available with either Protein G agarose or Protein G magnetic beads and contain all buffers and reagents needed to perform up to 30 ChIP assays.

Each kit is designed to optimize:

❶ Chromatin Integrity

Enzymatic digestion gently fragments the chromatin, protecting the integrity of the protein and the DNA.

❷ Assay Reliability

The Histone H3 antibody is a universal control for tracking assay efficiency and reagent performance.

❸ Antibody Specificity

CST™ ChIP-validated antibodies are rigorously tested and validated, ensuring they will specifically bind to their intended target.

SimpleChIP® Plus Enzymatic Chromatin IP Kits

No.	Name	Application	Reactivity
#9004	SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads)	ChIP	H, M, R, Mk
#9005	SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads)	ChIP	H, M, R, Mk

Cell Signaling TECHNOLOGY®

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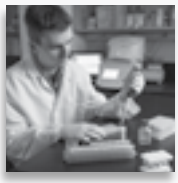

FRONT COVER PHOTO:
Curtis, Research Associate,
has been with CST since 2005.

Hongying, Research Associate,
has been with CST since 2006.

FOUNDED BY RESEARCH SCIENTISTS IN 1999, Cell Signaling Technology (CST) is a private, family-owned company with over 400 employees worldwide. Active in the field of applied systems biology research, particularly as it relates to cancer, CST understands the importance of using antibodies with high levels of specificity and lot-to-lot consistency. It's why we produce all of our antibodies in house, and perform painstaking validations for multiple applications. And the same CST scientists who produce our antibodies also provide technical support for customers, helping them design experiments, troubleshoot, and achieve reliable results. We do this because that's what we'd want if we were in the lab. Because, actually, we are.

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ISO 9001:2008 certified