



Cell Signaling
TECHNOLOGY®

Antibodies and SimpleChIP® Reagents for
**Chromatin
Immunoprecipitation**

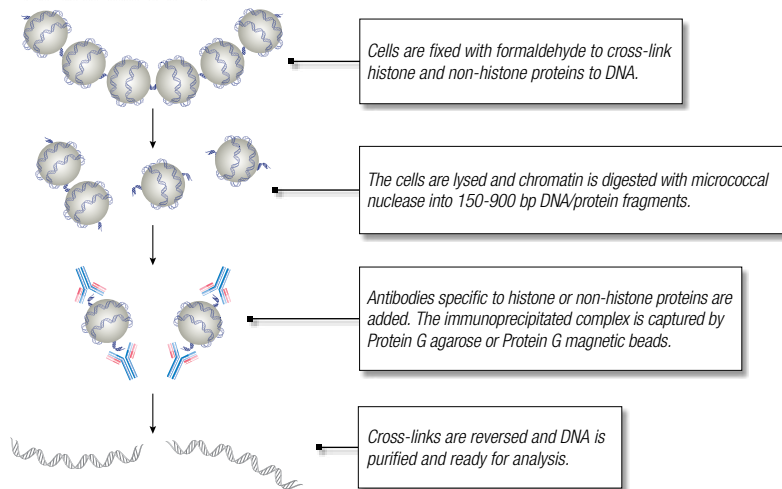
www.cellsignal.com



UNPARALLELED PRODUCT QUALITY, VALIDATION, AND TECHNICAL SUPPORT

SimpleChIP® Chromatin IP Kits

SimpleChIP® Enzymatic Chromatin IP Kits from Cell Signaling Technology (CST) are co-developed by CST and New England Biolabs scientists and contain the highest quality research reagents. 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. The same reagents in these kits have been used for in-house ChIP validation at CST, which will simplify your optimization. The kits can be utilized with any ChIP-validated antibody to detect endogenous levels of protein-DNA interactions and histone modifications in mammalian cells.

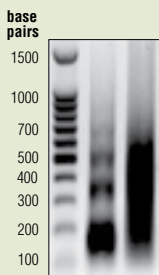


In a traditional ChIP assay, the identity of the DNA is determined by standard or quantitative PCR using primers specific for a known gene. Alternatively, the ChIP assay can be combined with sequencing (ChIP-seq), genomic tiling micro-array (ChIP on chip) techniques, or cloning strategies that allow for genome-wide analysis of protein-DNA interactions and histone modifications.

The SimpleChIP® Kit Advantage

Enzyme-based Chromatin Digestion vs. Sonication-based Chromatin Fragmentation

Prior to performing chromatin IP, it is important to process chromatin to the appropriate DNA fragment size. CST™ SimpleChIP® Enzymatic Chromatin IP Kits use micrococcal nuclease digestion to obtain chromatin fragments, whereas many other commercially available kits use a sonication-based method.



1 2

	SimpleChIP® Kits from Cell Signaling Technology	Competitor Kits
Fragmentation Method	Enzymatic	Sonication
Chromatin Quality	High (mild sample treatment preserves chromatin integrity and antibody epitopes)	Low (rigorous sample treatment disrupts chromatin integrity and antibody epitopes)
IP Efficiency	High	Low
Detection	Higher sensitivity (especially crucial for transcription factors and cofactors)	Lower sensitivity (significantly decreased signal for less abundant chromatin binding proteins)

< **Enzyme-based and sonication-based ChIP kits produce chromatin fragments of a similar size, but differing chromatin integrity.** Chromatin was prepared from 4×10^7 HCT 116 human colorectal carcinoma cells according to the protocols included with the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 and from 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.

SimpleChIP® Enzymatic Chromatin IP Kits

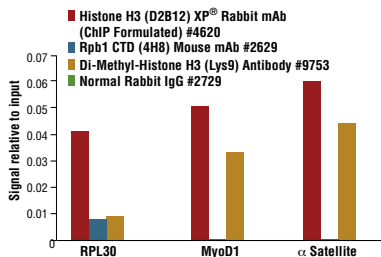
30 assays

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

Glycine Solution (10X)	Normal Rabbit IgG #2729
Buffer A (4X)	ChIP-Grade Protein G Agarose Beads (blocked with BSA and sonicated salmon sperm DNA)
Buffer B (4X)	DNA Spin Columns
ChIP Buffer (10X)	Protease Inhibitor Cocktail (200X)
ChIP Elution Buffer (2X)	Proteinase K
5 M NaCl, 0.5 M EDTA	SimpleChIP® Human RPL30 Exon 3 Primers
DNA Binding Reagent A	SimpleChIP® Mouse RPL30 Intron 2 Primers
DNA Wash Reagent B	Histone H3 (D2B12) XP® Rabbit mAb (ChIP Formulated) #4620
DNA Elution Reagent C	
RNAse A (10 mg/ml)	
Micrococcal Nuclease	1M DTT

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

This kit contains the same components as #9002 except #9003 contains ChIP-Grade Protein G Magnetic Beads (blocked with BSA).

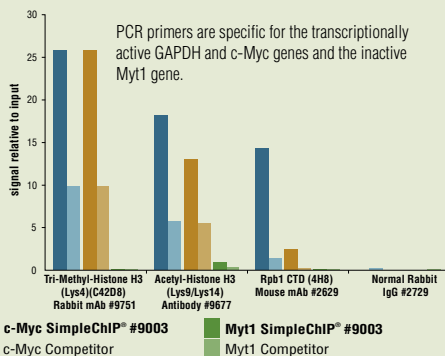
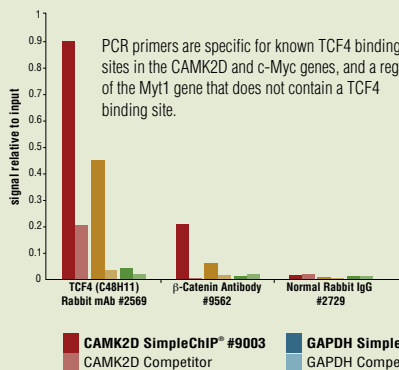


SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads)

#9003: Chromatin IPs were performed using digested chromatin from HeLa cells and the indicated ChIP validated antibodies. Purified DNA was analyzed by quantitative real-time PCR, using SimpleChIP® Human RPL30 Exon 3 Primers #7014 (control primer set), SimpleChIP® Human MyoD1 Exon 1 Primers #4490, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).

Product References

Bommer, G. T. et al. (2010). <i>J. Biol. Chem.</i> 285, 1928-1938.	Xu, Y. et al. (2010). <i>J. Cell Biol.</i> 191, 31-43.	Bonzo, J. A. et al. (2012). <i>J. Biol. Chem.</i> 287, 7345-7356.
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Kong, D. K. et al. (2010). <i>Mol. Biol. Cell</i> 21, 1335-1349.	Kizuka, Y. et al. (2011). <i>J. Biol. Chem.</i> 286, 31875-31884.	Owens, P. et al. (2012). <i>Proc. Natl. Acad. Sci. U.S.A.</i> 109, 2814-2819.
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Onishi, Y. et al. (2010) <i>BioSci. Rep.</i> 31, 57-62.	Ouyang, J. et al. (2011). <i>Blood</i> 117, 4315-4322.	
	Textor, S. et al. (2011). <i>Cancer Res.</i> 71, 5998-6009.	



SimpleChIP® digested chromatin is more conducive to immunoprecipitation than sonicated chromatin. Chromatin immunoprecipitations were performed with 10 µg of cross-linked HCT 116 chromatin and the indicated antibodies, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 and 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. For every target tested, enzyme-digested chromatin showed better enrichment of target DNA loci than did sonicated chromatin.

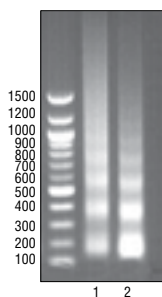
New SimpleChIP® Plus Chromatin IP Kits

For Use with Tissue Samples or Cultured Cells

New SimpleChIP® Plus Chromatin IP Kits from Cell Signaling Technology (CST) are optimized to detect endogenous levels of protein-DNA interactions in both cultured cells and tissue samples. These kits contain all reagents necessary to perform enzymatic digestion-based chromatin immunoprecipitation (ChIP) experiments quickly and easily from cells or tissue samples, as well as positive and negative controls that ensure confidence in your results.

The benefits of SimpleChIP® Plus Kits include:

- A detailed protocol optimized for analysis of cells or tissue samples is provided, saving your valuable time and reagents.
- Kits contain all necessary beads, buffers, and controls, providing an economical alternative to purchasing components individually.
- These kits are used in-house for validation of our ChIP-recommended antibodies, saving you the trouble of optimization.
- Technical support is provided by the same scientists who developed and produce the kits, ensuring a thorough, fast, and accurate response.

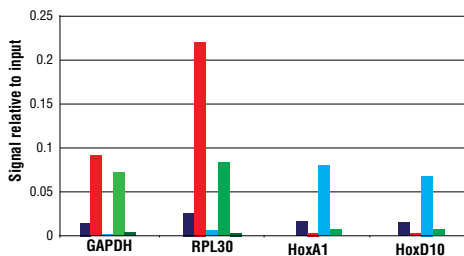


		Reactivity
#9004	SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads)	H, M, R, Mk
#9005	SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads)	H, M, R, Mk

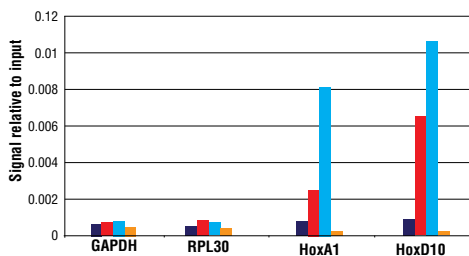
SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads) #9004:

Mouse brain and mouse liver tissue were prepared and disaggregated into a single-cell suspension using a Dounce homogenizer and tissue disaggregator, respectively. The cells were then formaldehyde-crosslinked and chromatin was prepared and digested. DNA was purified and separated by electrophoresis on a 1% agarose gel and stained with ethidium bromide. The majority of chromatin from both brain (lane 1) and liver (lane 2) was digested to 1 to 5 nucleosomes in length (150 to 900 bp).

- Histone H3 (D2B12) XP® Rabbit mAb (ChIP Formulated) #4620
- Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751
- Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733
- Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb #9649
- Rpb1 CTD (4H8) Mouse mAb #2629
- Normal Rabbit IgG #2729

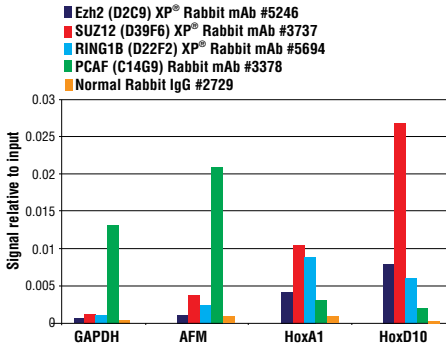
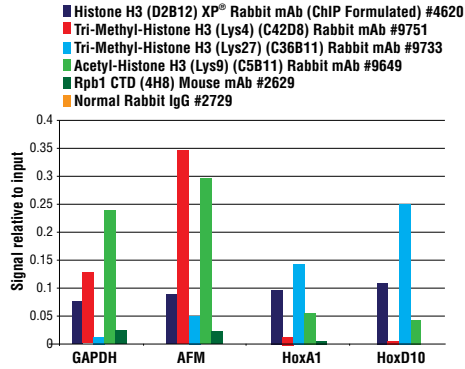


- Ezh2 (D2C9) XP® Rabbit mAb #5246
- SUZ12 (D39F6) XP® Rabbit mAb #3737
- RING1B (D22F2) XP® Rabbit mAb #5694
- Normal Rabbit IgG #2729

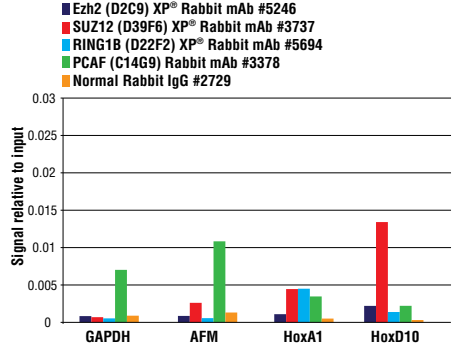
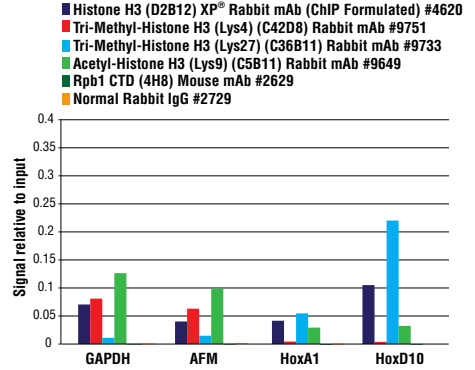


SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads) #9004: Mouse brain was prepared and disaggregated into a single-cell suspension using a Dounce homogenizer. The cells were then cross-linked with formaldehyde, and chromatin was prepared and digested. Chromatin immunoprecipitations were performed using the indicated ChIP-validated antibodies. Purified DNA was analyzed by quantitative real-time PCR using SimpleChIP® Mouse GAPDH Intron 2 Primers #8986, SimpleChIP® Mouse RPL30 Intron 2 Primers #7015, SimpleChIP® Mouse HoxA1 Promoter Primers #7341, and SimpleChIP® Mouse HoxD10 Intron 1 Primers #7429. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to 1).

A SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic beads) #9005



B Competitor's Sonication-based Tissue ChIP Kit



SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic beads) #9005 (A) compared to a competitor's sonication-based Tissue Chromatin IP kit (B). Mouse liver was prepared and disaggregated into a single-cell suspension using a tissue disaggregator. The cells were then cross-linked with formaldehyde, and chromatin was prepared and digested according to manufacturer's recommendations. Chromatin immunoprecipitations were performed using the indicated ChIP-validated antibodies. Purified DNA was analyzed by quantitative real-time PCR using SimpleChIP® Mouse GAPDH Intron 2 Primers #8986, SimpleChIP® Mouse AFM Intron 2 Primers #7269, SimpleChIP® Mouse HoxA1 Promoter Primers #7341, and SimpleChIP® Mouse HoxD10 Exon 1 Primers #7429. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to 1).

Comparison of immunoprecipitated DNA relative to input chromatin (signal relative to input) obtained using the SimpleChIP® Plus Chromatin IP Kit #9005 to that obtained using the competitor kit demonstrates the increased immuno-enrichment and superior sensitivity of the SimpleChIP® Plus Kit.

Over 140 ChIP Validated Antibodies

ChIP recommended antibodies from Cell Signaling Technology (CST) have been validated in-house by our antibody development scientists using the same rigorous standards as all other recommended applications. Technical support is provided by the same scientists that validate the antibody, which ensures a fast, thorough, and accurate response. Visit our website for the most up-to-date listing of all ChIP-validated antibodies.

ChIP Validated Antibodies		Reactivity	Positive Control Primer	Negative Control Primer
	#9814 Acetylated-Lysine (Ac-K2-100) Rabbit mAb	all species expected	#4471 H, #5516 H	#4486 H, #4493 H
	#9441 Acetylated-Lysine Antibody	all species expected	#5516 H, #7014 H, #7015 M	#4490 H, #4493 H
XP	#6964 Bmi1 (D20B7) XP® Rabbit mAb	H, Mk	#7707 H, #5517 H	#4486 H
	#5856 Bmi1 (D42B3) Rabbit mAb	H, M, R, Mk	#7707 H, #5517 H	#4486 H
New	#8814 Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb	H, M, R, Mk, (C, X, Z, B, Dg, Pg)	#8973 H, #5111 H	#4486 H
	#9562 β-Catenin Antibody	H, M, R, Mk, (Z)	#8973 H, #5111 H	#4486 H
New XP	#8480 β-Catenin (D10A8) XP® Rabbit mAb	H, M, R, Mk, (Z, B, Pg)	#8973 H, #5111 H	#4486 H
	#9581 β-Catenin Antibody (Amino-terminal Antigen)	H, M, R, Mk	#5111 H	#4486 H
	#9587 β-Catenin Antibody (Carboxy-terminal Antigen)	H, M, R, Mk, (C, X, B, Dg, Pg)	#8973 H, #5111 H	#4486 H
	#4771 Acetyl-CBP (Lys1535)p300 (Lys1499) Antibody	H, M, R, Mk	#4829 H	#4486 H
New	#7389 CBP (D6C5) Rabbit mAb	H, M, R, Mk	#4829 H	#4486 H
New	#7425 CBP (D9B6) Rabbit mAb	H, M, R, Mk	#4829 H	#4486 H
New	#4351 CHD1 (D8C2) Rabbit mAb	H, M, R, Mk, (B, Dg, Pg)	#4653 M, #7015 M	#8985 M
	#9198 Phospho-CREB (Ser133) (87G3) Rabbit mAb	H, M, R	#4829 H	#4486 H
	#4276 Phospho-CREB (Ser133) (D1G6) Rabbit mAb	H, M, R	#4829 H	#4486 H
	#9197 CREB (48H2) Rabbit mAb	H, M, R, Mk, Dm	#4829 H	#4486 H
	#4820 CREB (D76D11) Rabbit mAb	H, M, R, Hm, Mk, Dm	#4829 H	#4486 H
XP	#3417 CTCF (D1A7) XP® Rabbit mAb	H, R, Mk, (B)	#5172 H	#4486 H
XP	#3418 CTCF (D31H2) XP® Rabbit mAb	H, M, R, Mk, (B)	#5172 H	#4486 H
	#2899 CTCF Antibody	H, M, R, Mk	#5172 H	#4486 H
	#3742 E2F-1 Antibody	H, (R)	#7001 H	#4486 H
	#4153 EGR1 (15F7) Rabbit mAb	H, M, R, (B)	#5549 H	#4486 H
	#4154 EGR1 (44D5) Rabbit mAb	H, M, R, (B)	#5549 H	#4486 H
New	#8644 Estrogen Receptor α (D8H8) Rabbit mAb	H	#9673 H, #9702 H	#4486 H
XP	#5246 Ezh2 (D2C9) XP® Rabbit mAb	H, M, R, Mk	#7707 H, #5517 H	#4486 H
	#4905 Ezh2 Antibody	H, M, R, Pg	#7707 H, #5517 H	#4486 H
	#2251 FosB (5G4) Rabbit mAb	H, M, R	#7983 R	#7964 R
XP	#5348 Phospho-c-Fos (Ser32) (D82C12) XP® Rabbit mAb	H, M, R, (Hm, Mk, B, Pg)	#7983 R	#7964 R
	#2250 c-Fos (9F6) Rabbit mAb	H, M, R, (Hm, B, Pg)	#7983 R	#7964 R
	#5841 Phospho-FRA1 (Ser265) (D22B1) Rabbit mAb	H, M, R, (Mk, B)	#7983 R	#7964 R
New XP	#3660 Glucocorticoid Receptor (D8H2) XP® Rabbit mAb	H, M, R, Mk	#7981 H	#4486 H
XP	#8240 Ubiquityl-Histone H2A (Lys119) (D27C4) XP® Rabbit mAb	H, M, R, Mk	#7707 H, #5517 H	#4486 H, #5516 H
New	#9072 Acetyl-Histone H2B (Lys12) (D7H4) Rabbit mAb (ChIP Formulated)	H, (M, R, Mk, C, X, Z, B, Pg)	#5516 H, #7014 H	#4490 H, #4486 H
New XP	#9083 Acetyl-Histone H2B (Lys15) (D8H1) XP® Rabbit mAb	H, M, R, Mk, (Z, B, Pg)	#7014 H	#4486 H
New XP	#5546 Ubiquityl-Histone H2B (Lys120) (D11) XP® Rabbit mAb	H, M, R, Mk	#5047 H, #4478 H	#5037 H, #4471 H
XP	#5326 Mono-Methyl-Histone H3 (Lys4) (D1A9) XP® Rabbit mAb	H, M, R, Mk	#5047 H	#5037 H
	#9725 Di-Methyl-Histone H3 (Lys4) (C64G9) Rabbit mAb	H, M, R, Mk	#7014 H, #7015 M	#4486 H, #4490 H
	#9726 Di-Methyl-Histone H3 (Lys4) Antibody	H, M, R, Mk, (X, Z)	#7014 H, #7015 M	#4486 H, #4490 H
	#9751 Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb	H, M, R, Mk, Dm, Sc, (X, Z)	#5516 H, #7014 H, #7015 M	#4486 H, #4490 H
	#9727 Tri-Methyl-Histone H3 (Lys4) Antibody	H, M, R, Mk, (X, Z)	#5516 H, #7014 H, #7015 M	#4486 H, #4490 H
XP	#4658 Di-Methyl-Histone H3 (Lys9) (D85B4) XP® Rabbit mAb	H, M, R, Mk, (Dm, X, Z, B, Pg, Sc)	#5098 H, #4486 H	#4471 H, #7014 H
	#9753 Di-Methyl-Histone H3 (Lys9) Antibody	H, M, R, Mk, Dm, Sc	#5098 H, #4486 H	#5516 H, #7014 H
	#5327 Di/Tri-Methyl-Histone H3 (Lys9) (6F12) Mouse mAb	H, M, R, Mk	#5098 H, #4486 H	#5516 H
XP	#4473 Pan-Methyl-Histone H3 (Lys9) (D54) XP® Rabbit mAb	H, M, R, Mk, (C, Dm, X, Z, B, Pg, Sc)	#5098 H, #4486 H, #5077 H	#4471 H, #7014 H
	#4069 Pan-Methyl-Histone H3 (Lys9) Antibody	H, M, R, Mk, Z	#5098 H, #4486 H, #5077 H	#4471 H, #7014 H

ChIP Validated Antibodies		Reactivity	Positive Control Primer	Negative Control Primer
XP	#9728 Di-Methyl-Histone H3 (Lys27) (D18C8) XP [®] Rabbit mAb	H, M, R, Mk	#5098 H, #4490 H	#5516 H, #7014 H
	#9733 Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb	H, M, R, Mk, (X, Z)	#4490 H, #4493 H	#7014 H
	#9756 Tri-Methyl-Histone H3 (Lys27) Antibody	H, M, R, Mk, (X)	#4490 H, #4493 H	#5516 H, #7014 H
XP	#4909 Tri-Methyl-Histone H3 (Lys36) (D5A7) XP [®] Rabbit mAb	H, M, R, Mk, (Hm, C, Dm, X, Z, B)	#5047 H, #4478 H	#5037 H, #4471 H
XP	#5427 Di-Methyl-Histone H3 (Lys79) (D15E8) XP [®] Rabbit mAb	H, M, R, Mk	#5047 H	#5037 H
	#9649 Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb	H, M, R, Mk, Z, (Sc)	#5516 H, #7014 H, #7015 M	#4486 H, #4490 H
	#9671 Acetyl-Histone H3 (Lys9) Antibody	H, M, R, Mk, Dm, Sc	#5516 H, #7014 H, #7015 M	#5098 H, #4486 H
	#9677 Acetyl-Histone H3 (Lys9/Lys14) Antibody	H, M, R, Mk, (Z)	#7014 H, #7015 M	#4486 H, #4490 H
New	#7627 Acetyl-Histone H3 (Lys14) (D4B9) Rabbit mAb	H, M, R, Mk, (Hm, Dm, X, Z, Pg, Sc)	#7014 H, #7015 M	#4486 H, #4490 H
	#5275 Acetyl-Histone H3 (Lys14) Antibody (ChIP Formulated)	H, (M, R, Mk)	#4478 H, #7014 H, #7015 M	#4486 H, #4490 H
	#9675 Acetyl-Histone H3 (Lys18) Antibody	H, M, R	#5516 H, #7014 H, #7015 M	#4486 H, #4490 H
New XP	#8173 Acetyl-Histone H3 (Lys27) (D5E4) XP [®] Rabbit mAb	H, M, R, Mk, (Hm, X, Z)	#5516 H, #7014 H, #7015 M	#5098 H, #4486 H
	#4353 Acetyl-Histone H3 (Lys27) Antibody	H, M, R, Mk, (Hm, C, Dm, X, Z, B)	#5516 H, #7014 H, #7015 M	#5098 H, #4486 H
XP	#4620 Histone H3 (D2B12) XP [®] Rabbit mAb (ChIP Formulated)	H, M, (R, Hm, Mk, C, Dm, X, Z, B)	#4486 H, #7014 H, #7015 M	-
	#2650 Histone H3 Antibody (ChIP Formulated)	H, M, (R, Mk, C, Dm, X, Z, B)	#4486 H, #7014 H, #7015 M	-
New	#8647 Acetyl-Histone H4 (Lys5) (D12B3) Rabbit mAb	H, M, R, Mk, (C, Dm, X, Z, B, Pg, Ce)	#5516 H, #7014 H	#4490 H, #4486 H
	#9672 Acetyl-Histone H4 (Lys5) Antibody	H, M, R, Mk, (C, Dm, X, Z, B, Pg)	#7014 H	#4486 H
	#5737 Tri-Methyl-Histone H4 (Lys20) (D84D2) Rabbit mAb	H, M, R, Mk, (X, B, Pg)	#5098 H, #4486 H	#5516 H
	#2960 Histone H4 (L64C1) Mouse mAb (ChIP Formulated)	H, (M, R, Mk, Dm, X, Z, B)	#4486 H, #4490 H	-
	#4356 HSF1 Antibody	H, M, R, Mk	#5551 H	#4486 H
	#4299 IRF-4 (D43H10) Rabbit mAb	H, R	#5156 H	#4486 H
	#4964 IRF-4 Antibody	H	#5156 H	#4486 H
	#5628 IRF-8 (D20D8) Rabbit mAb	H, M, (R, Mk, X, B)	#9013 H	#4486 H
	#3753 JunB (C37F9) Rabbit mAb	H, M, R, Mk	#7983 R	#7964 H
New XP	#3270 Phospho-c-Jun (Ser73) (D47G9) XP [®] Rabbit mAb	H, M, R, Mk	#7983 R	#7964 H
	#9165 c-Jun (60A8) Rabbit mAb	H, M, R, Mk	#4829 H	#4486 H
XP	#3619 MCM2 (D7G11) XP [®] Rabbit mAb	H, M, R, Mk	#5148 H	#4486 H
	#5415 Mouse (G3A1) mAb IgG1 Isotype Control		-	-
	#9402 c-Myc Antibody	H, M, R, Pg	#4779 H	#4486 H
New XP	#8822 Nanog (D2A3) XP [®] Rabbit mAb (Mouse Specific)	M	#4653 M, #4659 M	#7015 M
XP	#5232 Nanog (D73G4) XP [®] Rabbit mAb (ChIP Formulated)	H	#4641 H, #4649 H	#4486 H
New	#8785 Nanog (D1G10) Rabbit mAb (Mouse Specific; ChIP Formulated)	M	#4653 M, #4659 M	#7015 M
	#3580 Nanog Antibody	H	#4641 H, #4649 H	#4486 H
	#4373 NeuroD (D35G2) Rabbit mAb	H, M, R	#5549 H	#4486 H
	#3035 NF-κB1 p105/p50 Antibody	H, Mk	#5552 H	#4486 H
New XP	#8242 NF-κB p65 (D14E12) XP [®] Rabbit mAb	H, M, R, Hm, Mk, Dg	#5552 H	#4486 H
New	#6956 NF-κB p65 (L8F6) Mouse mAb	H, M, R, Hm, Mk, Mi, B, Dg, Pg	#5552 H	#4486 H
	#4147 Cleaved Notch1 (Val1744) (D3B8) Rabbit mAb	H, M, R	#7273 H	#4486 H
XP	#3608 Notch1 (D1E11) XP [®] Rabbit mAb	H, M, R	#7273 H	#4486 H
	#3439 Notch1 (C37C7) Rabbit mAb	H	#7273 H	#4486 H
	#5677 Oct-4A (C30A3C1) Rabbit mAb (ChIP Formulated)	H, M	#4641 H, #4653 M, #4649 H, #4659 M	#4486 H, #7015 M
	#2890 Oct-4A (C52G3) Rabbit mAb	H	#4641 H, #4649 H	#4486 H
	#2750 Oct-4 Antibody	H, (Mk)	#4641 H, #4649 H	#4486 H
	#9284 Phospho-p53 (Ser15) Antibody	H, M, R, Mk	#6449 H	#4486 H
	#2527 p53 (7F5) Rabbit mAb	H, Mk	#6449 H	#4486 H
	#2524 p53 (1C12) Mouse mAb	H, M, R, Mk	#6449 H	#4486 H
	#9282 p53 Antibody	H, R, Mk	#6449 H	#4486 H
	#3378 PCAF (C14G9) Rabbit mAb	H, M, R, Mk, (B)	#4829 H	#4486 H
New XP	#8757 Progesterone Receptor A/B (D8Q2J) XP [®] Rabbit mAb	H, (Mk)	#7859 H	#4486 H
	#2258 PU.1 (9G7) Rabbit mAb	H, M, (Mk, Pg)	-	#4486 H

Reactivity Key: H=Human M=Mouse R=Rat Mk=Monkey Mi=Mink Pg=Pig Sc=S. cerevisiae C=Chicken Hm=Hamster B=Bovine Dm=D. melanogaster X=Xenopus Z=Zebrafish Dg=Dog Species enclosed in parentheses are predicted to react based on 100% sequence homology.

ChIP Validated Antibodies

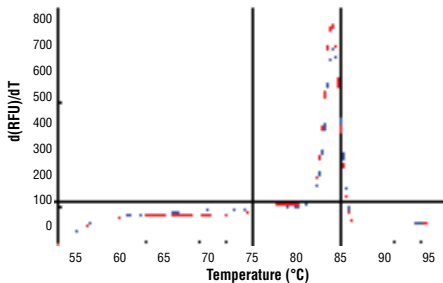
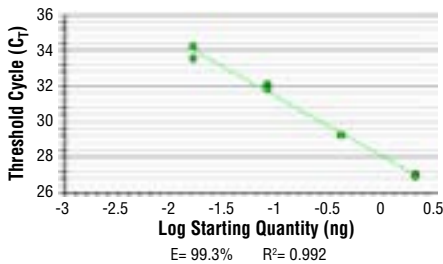
	Reactivity	Positive Control Primer	Negative Control Primer
	#2266 PU.1 Antibody	H, M, (Mk, Pg)	-
XP	#3900 Rabbit (DA1E) mAb IgG XP [®] Isotype Control	-	-
	#2729 Normal Rabbit IgG	-	-
	#9313 Rb (D20) Rabbit mAb	H, M, Mk	#7001 H
	#9309 Rb (4H1) Mouse mAb	H, Mk, B, Pg	#7001 H
	#2629 Rpb1 CTD (4H8) Mouse mAb	H, M, R, Mk, (Hm, Dm, Sc)	#4471 H, #5516 H
XP	#5694 RING1B (D22F2) XP [®] Rabbit mAb	H, M, R, Mk	#7707 H, #5517 H
XP	#5313 RBPSUH (D10A4) XP [®] Rabbit mAb	H, M, R, Mk	#7273 H
New	#7691 SIN3A (D1B7) Rabbit mAb	H, M, R, Mk, (Pg)	#9673 H, #5516 H
New	#8056 SIN3A (D9D6) Rabbit mAb	H, Mk	#9673 H, #5516 H
XP	#6944 Smad1 (D59D7) XP [®] Rabbit mAb	H, M, (Mk, X, B)	#5139 H
	#9743 Smad1 Antibody	H, M, Mk	#5139 H
	#9511 Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad8 (Ser426/428) Antibody	H, M, R, Mi, X	#5139 H
XP	#5339 Smad2 (D43B4) XP [®] Rabbit mAb	H, M, R, Mk	#5139 H, #4669 H
New	#8685 Smad2/3 (D7G7) XP [®] Rabbit mAb	H, M, R, Mk	#5139 H, #4669 H
XP	#5678 Smad2/3 Antibody	H, M, R, Mk, (X)	#5139 H, #4669 H
	#9520 Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb	H, M, R, (Mk, X, Z, B)	#5139 H, #4669 H
	#9523 Smad3 (C67H9) Rabbit mAb	H, M, R, Mk, (X, Z, B)	#5139 H, #4669 H
	#9515 Smad4 Antibody	H, M, R, Mk	#5139 H, #4669 H
XP	#5024 Sox2 (D6D9) XP [®] Rabbit mAb (ChIP Formulated)	H, (Mk, B, Dg)	#4641 H, #4649 H
	#2748 Sox2 Antibody	H, M, (R, Mk, B, Dg)	#4641 H, #4653 M, #4649 H, #4659 M
New	#9389 SP1 (D4C3) Rabbit mAb	H, Mk	#7531 H
XP	#5147 SRF (D71A9) XP [®] Rabbit mAb	H, M, R, Mk, Pg	#4663 H
	#9167 Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb	H, M	#5148 H
New	#7649 Phospho-Stat1 (Tyr701) (D4A7) Rabbit mAb	H, M, R, (Mk)	#5148 H
	#9171 Phospho-Stat1 (Tyr701) Antibody	H, M, R, (B, Dg)	#5148 H
New	#8826 Phospho-Stat1 (Ser727) (D3B7) Rabbit mAb	H, M, R, (Mk, B)	#5148 H
	#9177 Phospho-Stat1 (Ser727) Antibody	H, M, R, (B)	#5148 H
	#9172 Stat1 Antibody	H, M, R, Mk, (B, Dg)	#5148 H
XP	#9145 Phospho-Stat3 (Tyr705) (D3A7) XP [®] Rabbit mAb	H, M, R, Mk, (Hm, B, Pg)	#4663 H
	#9131 Phospho-Stat3 (Tyr705) Antibody	H, M, R, Mk, (C, B, Dg)	#4663 H
	#9134 Phospho-Stat3 (Ser727) Antibody	H, M, R, (B)	#4663 H
New	#8768 Stat3a (D1A5) XP [®] Rabbit mAb	H, M, R, Hm, Mk, (Pg)	#4663 H
XP	#4904 Stat3 (79D7) Rabbit mAb	H, M, R, Mk	#4663 H
	#9139 Stat3 (124H6) Mouse mAb	H, M, R, Mk	#4663 H
	#9132 Stat3 Antibody	H, M, R, Mk, (B)	#4663 H
New	#4134 Phospho-Stat4 (Tyr693) (D2E4) Rabbit mAb	H, (M, R, Mk, Pg)	#9014 H
	#5267 Phospho-Stat4 (Tyr693) Antibody	H, (M, R)	#9014 H
	#2653 Stat4 (C46B10) Rabbit mAb	H, M, R	#9014 H
	#9358 Stat5 (3H7) Rabbit mAb	H, M, R	#5131 M
	#9351 Phospho-Stat5 (Tyr694) Antibody	H, M, (R, B)	#5131 M
	#9363 Stat5 Antibody	H, M, R, Mk	#5131 M
New	#5397 Stat6 (D3H4) Rabbit mAb	H, M, R, (Mk, B, Dg, Pg)	#7710 H
XP	#3737 SUZ12 (D39F6) XP [®] Rabbit mAb	H, M, R, Mk	#7707 H, #5517 H
	#2569 TCF4 (C48H11) Rabbit mAb	H, (M, C)	#5111 H
	#2565 TCF4 (C9B9) Rabbit mAb	H, (M, R)	#5111 H

Reactivity Key: H=Human M=Mouse R=Rat Mk=Monkey Mi=Mink Pg=Pig Sc=S. cerevisiae C=Chicken Hm=Hamster B=Bovine Dm=D. melanogaster X=Xenopus Z=Zebrafish Dg=Dog Species enclosed in parentheses are predicted to react based on 100% sequence homology.

SimpleChIP® Control PCR Primers

SimpleChIP® Control PCR Primers featured throughout this brochure are a mix of forward and reverse primers which can be used to amplify DNA that has been isolated using ChIP. These primers amplify positive control DNA sequences that contain known binding sites of the target protein detected by the antibody employed in the ChIP assay, and can also be used as a negative control to demonstrate antibody specificity.

- Primers are designed, tested, and optimized in-house in conjunction with our ChIP validated antibodies and SimpleChIP® Kits, saving time and reagents.
- Primers are optimized for use in real-time PCR with SYBR® Green dye, which simplifies quantification of DNA enrichment.
- Technical Support is provided by the scientists who designed and use these products, and know them best.



SimpleChIP® Human Axin2 Intron 1 Primers #8973:

UPPER PANEL: SimpleChIP® Human Axin2 Intron 1 Primers were tested on DNA isolated from cross-linked cells using the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. Real-time PCR was performed in duplicate on a serial dilution of 2% total input DNA (20 ng, 4 ng, 0.8 ng, and 0.16 ng) using a real-time PCR detection system and SYBR® Green reaction mix. The PCR amplification efficiency (E) and correlation coefficient (R²) were calculated based on the corresponding threshold cycle (C_t) of each dilution sample during 40 cycles of real-time PCR (95°C denaturation for 15 sec, 65°C anneal/extension for 60 sec).

LOWER PANEL: PCR product melting curves were obtained for real-time PCR reactions performed using SimpleChIP® Human Axin2 Intron 1 Primers. Data is shown for both duplicate PCR reactions using 20 ng of total DNA. The melt curve consists of 80 melt cycles, starting at 55°C with increments of 0.5°C per cycle. Each peak is formed from the degradation of a single PCR product.

	Reactivity
#5098 SimpleChIP® Human AFM Intron 1 Primers	H
#7269 SimpleChIP® Mouse AFM Intron 2 Primers	M
#5047 SimpleChIP® Human γ-Actin Intron 3 Primers	H
#5037 SimpleChIP® Human γ-Actin Promoter Primers	H
#4486 SimpleChIP® Human α Satellite Repeat Primers	H
#8973 SimpleChIP® Human Axin2 Intron 1 Primers	H
#5111 SimpleChIP® Human CaMK2D Intron 3 Primers	H
#7983 SimpleChIP® Rat CCRN4L Promoter Primers	R
#5131 SimpleChIP® Mouse CIS Intron 1 Primers	M
#7531 SimpleChIP® Human DHFR Intron 1 Primers	H
#7710 SimpleChIP® Human DMD Intron 2 Primers	H
#5549 SimpleChIP® Human EGR1 Promoter Primers	H
#9673 SimpleChIP® Human ESR1 Promoter Primers	H
#7859 SimpleChIP® Human FKBP51 Intron 5 Primers	H
#4663 SimpleChIP® Human c-Fos Promoter Primers	H
#5516 SimpleChIP® Human GAPDH Exon 1 Primers	H
#4478 SimpleChIP® Human GAPDH Intron 2 Primers	H
#8986 SimpleChIP® Mouse GAPDH Intron 2 Primers	M
#4471 SimpleChIP® Human GAPDH Promoter Primers	H
#7964 SimpleChIP® Rat GAPDH Promoter Primers	R
#5550 SimpleChIP® Human GATA6 Promoter Primers	H
#7273 SimpleChIP® Human HES4 Promoter Primers	H
#7707 SimpleChIP® Human HoxA1 Intron 1 Primers	H
#7341 SimpleChIP® Mouse HoxA1 Promoter Primers	M
#5517 SimpleChIP® Human HoxA2 Promoter Primers	H
#7429 SimpleChIP® Mouse HoxD10 Exon 1 Primers	M
#5551 SimpleChIP® Human HSPA6 Promoter Primers	H
#5139 SimpleChIP® Human ID1 Promoter Primers	H
#5172 SimpleChIP® Human H19/Igf2 Imprinting Control Region Primers	H
#5552 SimpleChIP® Human IκBα Promoter Primers	H
#9013 SimpleChIP® Human MS4A7 Promoter Primers	H
#4490 SimpleChIP® Human MyoD1 Exon 1 Primers	H
#4493 SimpleChIP® Human MYT-1 Exon 1 Primers	H
#8985 SimpleChIP® Mouse MYT-1 Promoter Primers	M
#4779 SimpleChIP® Human NPM1 Intron 1 Primers	H
#4829 SimpleChIP® Human NR4A3 Promoter Primers	H
#4641 SimpleChIP® Human Oct-4 Promoter Primers	H
#4653 SimpleChIP® Mouse Oct-4 Promoter Primers	M
#4669 SimpleChIP® Human CDKN1A Intron 1 Primers	H
#6449 SimpleChIP® Human CDKN1A Promoter Primers	H
#8984 SimpleChIP® Mouse PITX3 Intron 1 Primers	M
#9014 SimpleChIP® Human PRF1 Promoter Primers	H
#9702 SimpleChIP® Human pS2 Promoter Primers	H
#7014 SimpleChIP® Human RPL30 Exon 3 Primers	H
#7015 SimpleChIP® Mouse RPL30 Intron 2 Primers	M
#5077 SimpleChIP® Human Sat2 Repeat Element Primers	H
#7681 SimpleChIP® Human SLC19A2 Promoter Primers	H
#4649 SimpleChIP® Human Sox2 Promoter Primers	H
#5156 SimpleChIP® Human SUB1 Promoter Primers	H
#5148 SimpleChIP® Human TAP1 Promoter Primers	H
#7001 SimpleChIP® Human Timeless Intron 1 Primers	H
#4659 SimpleChIP® Mouse XIST Intron 1 Primers	M

SimpleChIP® Assay Kits

10 assays

SimpleChIP® Assay Kits combine several ChIP validated antibodies with control PCR primer mixes that serve as markers for pluripotency or epigenetic status. Rigorous quality control at CST and in-house testing ensure the antibodies included in the kits meet the highest standards for quality, validation, and lot-to-lot consistency. The kits provide all reagents necessary to perform 10 ChIP assays per antibody and subsequent real-time PCR reactions. Pre-selected positive and negative primer sets are included in each kit, providing proven and appropriate controls for customer experiments.

#8980 SimpleChIP® Stem Cell Master Regulator Assay Kit

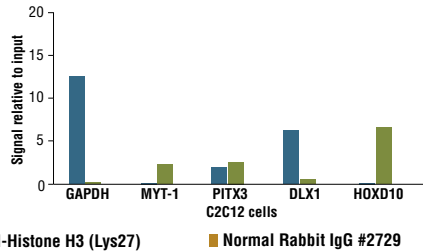
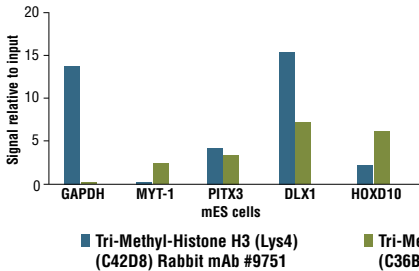
Nanog (D73G4) XP® Rabbit mAb (ChIP Formulated) #5232
Oct-4A (C30A3C1) Rabbit mAb (ChIP Formulated) #5677
Sox2 (D6D9) XP® Rabbit mAb (ChIP Formulated) #5024
SimpleChIP® Human Oct-4 Promoter Primers #4641
SimpleChIP® Human α Satellite Repeat Primers #4486

#8982 SimpleChIP® Human Bivalent Promoter Assay Kit

Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751
Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733
SimpleChIP® Human GAPDH Exon 1 Primers #5516
SimpleChIP® Human MYT-1 Exon 1 Primers #4493
SimpleChIP® Human GATA6 Promoter Primers #5550

#8981 SimpleChIP® Mouse Bivalent Promoter Assay Kit

Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751
Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733
SimpleChIP® Mouse GAPDH Intron 2 Primers #8986
SimpleChIP® Mouse MYT-1 Promoter Primers #8985
SimpleChIP® Mouse PITX3 Intron 1 Primers #8984



SimpleChIP® Mouse Bivalent Promoter Assay Kit #8981: Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 mES cells (left panel) or C2C12 cells (right panel) and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb, Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb, or 2 μ l of Normal Rabbit IgG, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Mouse GAPDH Intron 2 Primers #8986, SimpleChIP® Mouse MYT-1 Promoter Primers #8985, SimpleChIP® Mouse PITX3 Intron 1 Primers #8984, mouse DLX1 promoter primers, and mouse HOXD10 intron 1 primers. The amount of immunoprecipitated DNA in each sample is normalized for enrichment of total histone H3 and represented as signal relative to the total amount of input chromatin, which is equivalent to one. Note that the PITX3, DLX1 and HOXD10 promoters are all bivalent in stem cells, while only PITX3 remains bivalent in the differentiated cell line C2C12.

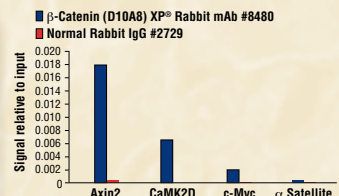
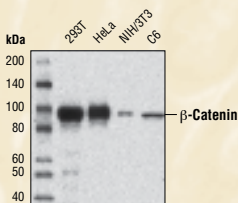
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SimpleChIP® Kit used with ChIP-Sequencing

Epigenetic landscapes consist of distinct domains of euchromatin and heterochromatin, which are unique to different cell types and different stages in development. Epigenetic regulation of gene expression is mediated through posttranslational modifications of histones. For example, tri-methylation of histone H3 Lys4 is a modification associated with active chromatin (euchromatin), while tri-methylation of histone H3 Lys27 is a mark of inactive heterochromatin.

A powerful method used to identify localized regions of histone modifications as well as binding sites for transcription factors on a genome-wide scale is the chromatin immunoprecipitation (ChIP) assay. Following immunoprecipitation the precipitated DNA is purified and can be analyzed by hybridization to oligonucleotide microarrays (ChIP-chip) or by high throughput DNA sequencing (ChIP-seq). These methods provide an opportunity to take a snapshot of DNA-protein interactions in a given cell type, using populations of cultured cells, subsets of cells taken at specific times of the cell cycle or development, or even cells taken directly from tissue samples. ChIP-seq technology offers the ability to identify binding sites across the entire genome in a single sequencing run, which generates short sequence reads that are sufficient for accurate mapping of the enriched DNA fragments to their genomic location.

Advantages of SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) for ChIP-Sequencing:

- Enzymatic digestion of chromatin is milder than sonication and better preserves the integrity of the chromatin and antibody epitopes, which means increased IP efficiency.
- Increased IP efficiency means enhanced detection of protein-bound DNA loci.
- ChIP-Grade Protein G Magnetic beads do NOT contain a DNA blocking agent such as salmon sperm DNA, which means no contamination of downstream sequencing.
- ChIP-Grade Protein G Magnetic beads do NOT require centrifugation, which translates to easier sample manipulation and more complete washes.
- ChIP-Grade Protein G Magnetic beads provide greater sensitivity with lower background, which is essential when detecting DNA bound transcription factors and cofactors.

Figure 1



Using the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 from Cell Signaling Technology, we have performed ChIP-seq experiments to identify the epigenetic signatures of tri-methyl-histone H3 Lys4 and tri-methyl-histone H3 Lys27 histone marks in the K562 erythroleukemia cell line. Chromatin was prepared from K562 cells as described in the SimpleChIP® protocol (Figure 1) and immunoprecipitated with Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751 (lane 1), Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733 (lane 2), and Normal Rabbit IgG #2729 (lane 3) as a negative control. The enrichment of tri-methyl histone H3 Lys4 and tri-methyl histone H3 Lys27 at known binding sites was confirmed by standard polymerase chain reaction (Figure 1). The immunoprecipitated DNA was then used to prepare libraries for sequencing with the Illumina GA2 sequencing platform. Obtained sequences were mapped to UCSC Human Genome Assembly (HG18) and only uniquely mapped sequences were retained (Figure 2A). Enriched binding sites are reflected by elevated number of sequence reads giving rise to peaks (Figure 2B and 2C). The rabbit monoclonal antibodies were found to give excellent enrichment, as reflected in the average peak height of 17 sequence reads, and very low background.

Figure 2A

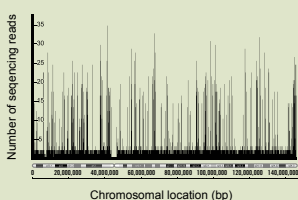


Figure 2B

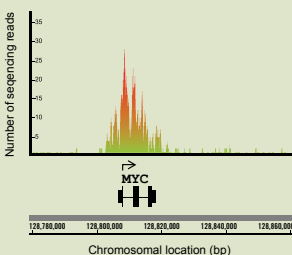
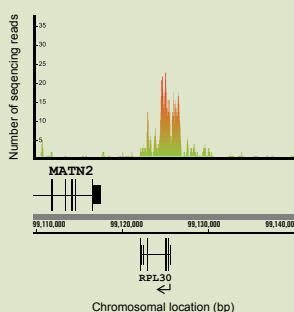


Figure 2C



CST would like to thank H. O'Geen and P.J. Farnham of the University of California, Los Angeles, California for sharing their ChIP-Sequencing data.



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