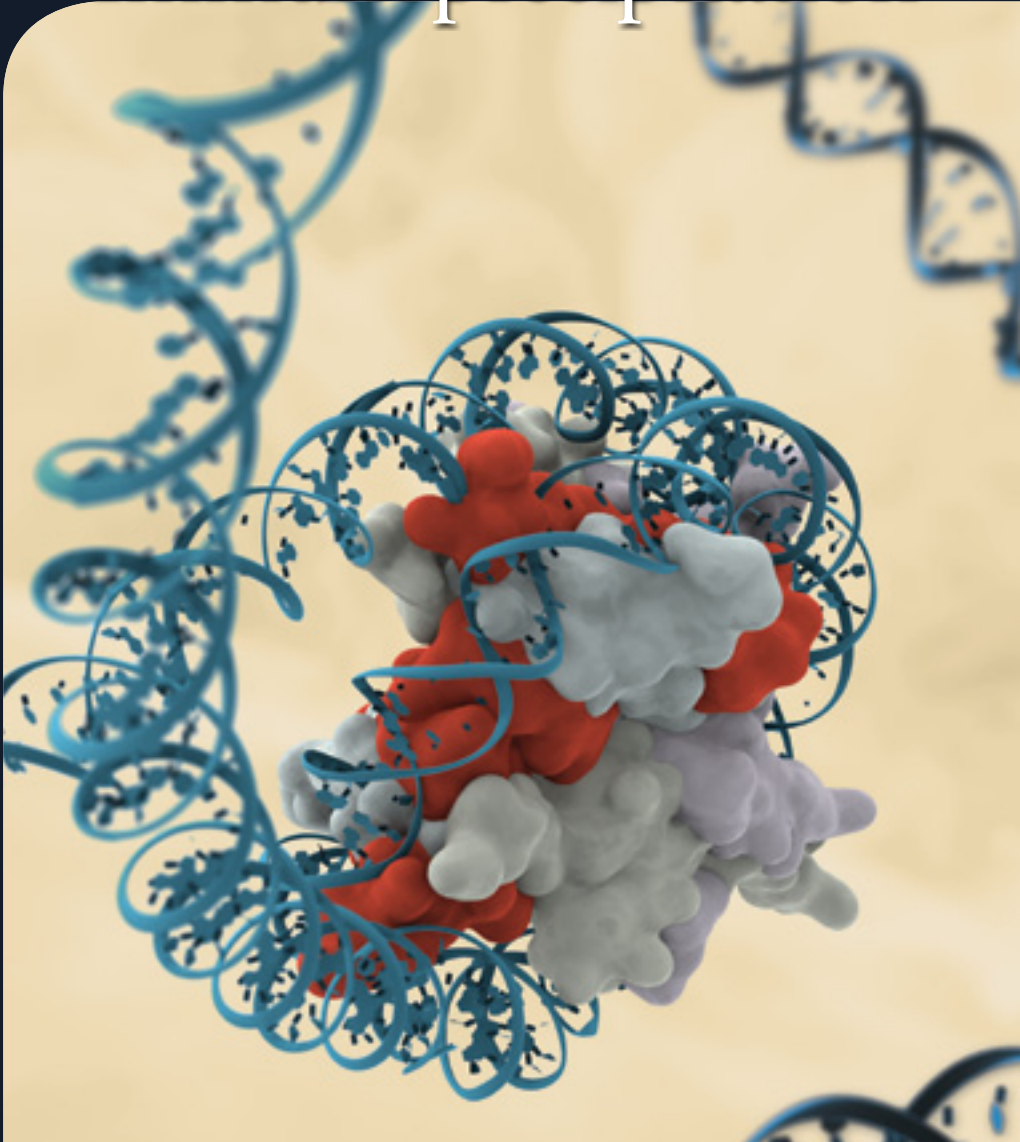




Cell Signaling
TECHNOLOGY®

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

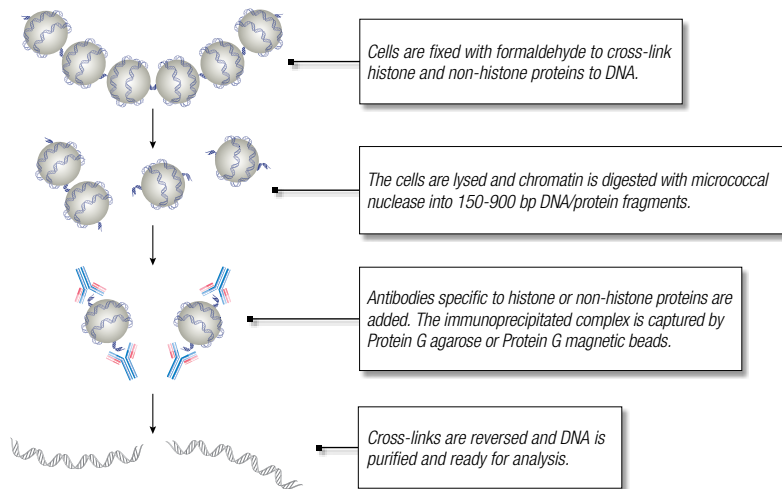
www.cellsignal.com



UNPARALLELED PRODUCT QUALITY, VALIDATION, AND TECHNICAL SUPPORT

SimpleChIP® Chromatin IP Kits

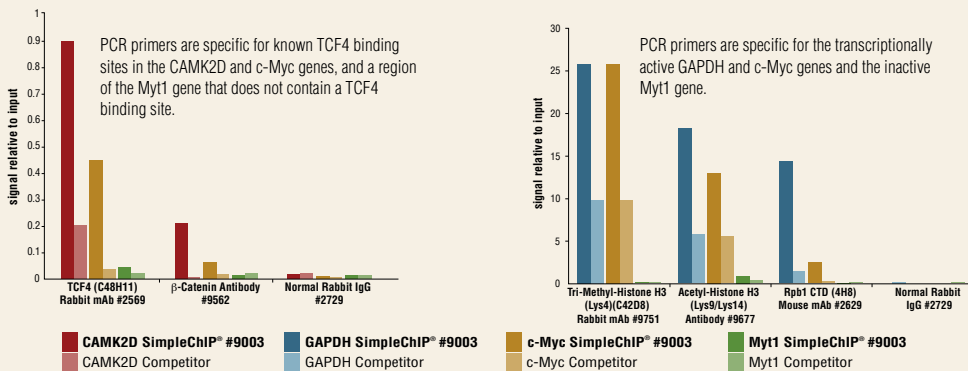
SimpleChIP® Enzymatic Chromatin IP Kits from Cell Signaling Technology (CST) are co-developed by CST and New England Biolabs scientists and contain the highest quality research reagents. These kits are available with either Protein G agarose or Protein G magnetic beads and contain all buffers and reagents 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 genomic tiling micro-array (ChIP on chip) techniques, sequencing (ChIP-seq), or cloning strategies that allow for genome-wide analysis of protein-DNA interactions and histone modifications.

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 competitor kits use a sonication-based method.



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.

SimpleChIP® Enzymatic Chromatin IP Kits

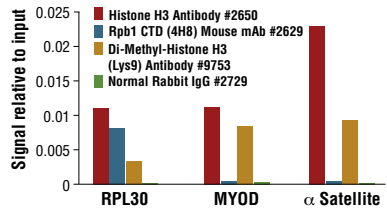
30 assays

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

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

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.

500 µl (250 PCR reactions)

#5047	SimpleChIP® Human γ-Actin Intron 3 Primers
#5037	SimpleChIP® Human γ-Actin Promoter Primers
#5098	SimpleChIP® Human AFM Intron 1 Primers
#5111	SimpleChIP® Human CaMK2D Intron 3 Primers
#4669	SimpleChIP® Human CDKN1A Intron 1 Primers
#5131	SimpleChIP® Mouse CIS Intron 1 Primers
#5549	SimpleChIP® Human EGR1 Promoter Primers
#4663	SimpleChIP® Human c-Fos Promoter Primers
#5516	SimpleChIP® Human GAPDH Exon 1 Primers
#4478	SimpleChIP® Human GAPDH Intron 2 Primers
#4471	SimpleChIP® Human GAPDH Promoter Primers
#8986	SimpleChIP® Mouse GAPDH Intron 2 Primers
#5550	SimpleChIP® Human GATA6 Promoter Primers
#5517	SimpleChIP® Human HoxA2 Promoter Primers
#5551	SimpleChIP® Human HSPA6 Promoter Primers
#5139	SimpleChIP® Human ID1 Promoter Primers
#5552	SimpleChIP® Human IκBα Promoter Primers
#5172	SimpleChIP® Human H19/Igf2 Imprinting Control Region Primers

#9013	SimpleChIP® Human MS4A7 Promoter Primers
#4490	SimpleChIP® Human MyoD1 Exon 1 Primers
#4493	SimpleChIP® Human MYT-1 Exon 1 Primers
#8985	SimpleChIP® Mouse MYT-1 Promoter Primers
#4779	SimpleChIP® Human NPM1 Intron 1 Primers
#4829	SimpleChIP® Human NR4A3 Promoter Primers
#4641	SimpleChIP® Human Oct-4 Promoter Primers
#4653	SimpleChIP® Mouse Oct-4 Promoter Primers
#8984	SimpleChIP® Mouse PITX3 Intron 1 Primers
#9014	SimpleChIP® Human PRF1 Promoter Primers
#7014	SimpleChIP® Human RPL30 Exon 3 Primers
#7015	SimpleChIP® Mouse RPL30 Intron 2 Primers
#5077	SimpleChIP® Human Sat2 Repeat Element Primers
#4649	SimpleChIP® Human Sox2 Promoter Primers
#5156	SimpleChIP® Human SUB1 Promoter Primers
#5148	SimpleChIP® Human TAP1 Promoter Primers
#7001	SimpleChIP® Human Timeless Intron 1 Primers
#4486	SimpleChIP® Human α Satellite Repeat Primers
#4659	SimpleChIP® Mouse XIST Intron 1 Primers

Over 100 ChIP Validated Antibodies

ChIP recommended antibodies from Cell Signaling Technology 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)	#4493 (H), #4486 (H)
#6964 Bmi1 (D20B7) XP® Rabbit mAb	H, Mk	#5517 (H)	#4486 (H)
#5856 Bmi1 (D42B3) Rabbit mAb	H, M, R, Mk	#5517 (H)	#4486 (H)
#9562 β-Catenin Antibody	H, M, R, Mk, (Z)	#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)	#5111 (H)	#4486 (H)
#4771 Acetyl-CBP (Lys1535)/p300 (Lys1499) Antibody	H, M, R, Mk	#4829 (H)	#4486 (H)
#4276 Phospho-CREB (Ser133) (D1G6) Rabbit mAb	H, M, R	#4829 (H)	#4486 (H)
#9198 Phospho-CREB (Ser133) (87G3) Rabbit mAb	H, M, R	#4829 (H)	#4486 (H)
#4820 CREB (D76D11) Rabbit mAb	H, M, R, Hm, Mk, Dm	#4829 (H)	#4486 (H)
#9197 CREB (48H2) Rabbit mAb	H, M, R, Mk, Dm	#4829 (H)	#4486 (H)
#3417 CTCF (D1A7) XP® Rabbit mAb	H, R, Mk, (B)	#5172 (H)	#4486 (H)
#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, M, (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)
#5246 Ezh2 (D2C9) XP® Rabbit mAb	H, M, R, Mk	#5517 (H)	#4486 (H)
#4905 Ezh2 Antibody	H, M, R, Pg	#5517 (H)	#4486 (H)
#5546 Ubiquityl-Histone H2B (Lys120) (D11) XP® Rabbit mAb	H, M, R, Mk	#5047 (H), #4478 (H)	#5037 (H), #4471 (H)
#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)	#4490 (H), #4486 (H)
#9751 Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb	H, M, R, Mk, Dm, Sc, (X, Z)	#5516 (H), #7014 (H), #7015 (M)	#4490 (H), #4486 (H)
#9726 Di-Methyl-Histone H3 (Lys4) Antibody	H, M, R, Mk, (X, Z)	#7014 (H), #7015 (M)	#4490 (H), #4486 (H)
#9727 Tri-Methyl-Histone H3 (Lys4) Antibody	H, M, R, Mk, (X, Z)	#5516 (H), #7014 (H), #7015 (M)	#4490 (H), #4486 (H)
#4658 Di-Methyl-Histone H3 (Lys9) (D85B4) XP® Rabbit mAb	H, M, R, Mk, (Dm, X, Z, B, Pg, Sc)	#5098 (H), #4486 (H)	#7014 (H), #4471 (H)
#4473 Pan-Methyl-Histone H3 (Lys9) (D54) XP® Rabbit mAb	H, M, R, Mk, (C, Dm, X, Z, B, Pg, Sc)	#5098 (H), 5077 (H), #4486 (H)	#7014 (H), #4471 (H)
#9753 Di-Methyl-Histone H3 (Lys9) Antibody	H, M, R, Mk, Dm, Sc	#5098 (H), #4486 (H)	#5516 (H), #7014 (H)
#4069 Pan-Methyl-Histone H3 (Lys9) Antibody	H, M, R, Mk, Z	#5098 (H), 5077 (H), #4486 (H)	#7014 (H), #4471 (H)
#9733 Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb	H, M, R, Mk, (X, Z)	#4490 (H), #4493 (H)	#7014 (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)
#9756 Tri-Methyl-Histone H3 (Lys27) Antibody	H, M, R, Mk, (X)	#4493 (H), #4490 (H)	#5516 (H), #7014 (H)
#9649 Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb	H, M, R, Mk, Z, (Sc)	#5516 (H), #7014 (H), #7015 (M)	#4490 (H), #4486 (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)	#4490 (H), #4486 (H)
#5275 Acetyl-Histone H3 (Lys14) Antibody (ChIP Formulated)	H, (M, R, Mk)	#4478 (H), #7014 (H), #7015 (M)	#4490 (H), #4486 (H)
#9675 Acetyl-Histone H3 (Lys18) Antibody	H, M, R	#5516 (H), #7014 (H), #7015 (M)	#4490 (H), #4486 (H)
#9728 Di-Methyl-Histone H3 (Lys27) (D18C8) XP® Rabbit mAb	H, M, R, Mk	#4490 (H), #5098 (H)	#5516 (H), #7014 (H)
#5427 Di-Methyl-Histone H3 (Lys79) (D15E8) XP® Rabbit mAb	H, M, R, Mk	#5047 (H)	#5037 (H)
#5327 Di/Tri-Methyl-Histone H3 (Lys9) (6F12) Mouse mAb	H, M, R, Mk	#4486 (H), #5098 (H)	#5516 (H)
#9754 Tri-Methyl-Histone H3 (Lys9) Antibody	H, M, R, Mk, (Dm, Pg)	#4486 (H), 5077 (H), #5098 (H)	#5516 (H), #7014 (H)
#4909 Tri-Methyl-Histone H3 (Lys36) (D5A7) XP® Rabbit mAb	H, M, R, Mk, (Hm, C, Dm, X, Z, B)	#4478 (H), #5047 (H)	#4471 (H), #5037 (H)
#4620 Histone H3 (D2B12) XP® Rabbit mAb (ChIP Formulated)	H, M, (R, Hm, Mk, C, Dm, X, Z, B)	#7014 (H), #4486 (H), #7015 (M)	
#2650 Histone H3 Antibody (ChIP Formulated)	H, M, (R, Mk, C, Dm, X, Z, B)	#7014 (H), #4486 (H), #7015 (M)	
#9672 Acetyl-Histone H4 (Lys5) Antibody	H, M, R, Mk, (C, Dm, X, Z, B, Pg)	#7014 (H)	#4486 (H)
#2594 Acetyl-Histone H4 (Lys8) Antibody	H, M, R, Mk	#7014 (H), #7015 (M)	#4486 (H), #5098 (H)
#5737 Tri-Methyl-Histone H4 (Lys20) (D84D2) Rabbit mAb	H, M, R, Mk, (X, B, Pg)	#4486 (H), #5098 (H)	#5516 (H)
#2960 Histone H4 (L64C1) Mouse mAb (ChIP Formulated)	H, (M, R, Mk, Dm, X, Z, B)	#4490 (H), #4486 (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)

ChIP Validated Antibodies

	Reactivity	Positive Control Primer	Negative Control Primer
#5628 IRF-8 (D20D8) Rabbit mAb	H, M, (R, Mk, X, B)		#4486 (H)
#9441 Acetylated-Lysine Antibody	all species expected	#5516 (H), #7014 (H), #7015 (M)	#4490 (H), #4493 (H)
#3619 MCM2 (D7G11) XP [®] Rabbit mAb	H, M, R, Mk	#5148 (H)	#4486 (H)
#9402 c-Myc Antibody	H, M, R, Pg	#4779 (H)	#4486 (H)
#5232 Nanog (D73G4) XP [®] Rabbit mAb (ChIP Formulated)	H	#4641 (H), #4649 (H)	#4486 (H)
#3580 Nanog Antibody	H	#4641 (H), #4649 (H)	#4486 (H)
#4373 NeuroD (D35G2) Rabbit mAb	H, R, (M)	#5549 (H)	#4486 (H)
#6956 NF-κB p65 (L8F6) Mouse mAb	H, M, R, Mk, Mi, Hm, B, Pg, Dg	#5552 (H)	#4486 (H)
#3035 NF-κB1 p105/p50 Antibody	H, Mk	#5552 (H)	#4486 (H)
#5677 Oct-4A (C30A3C1) Rabbit mAb (ChIP Formulated)	H, M	#4641 (H), #4649 (H), 4653 (M), 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)
#3378 PCAF (C14G9) Rabbit mAb	H, M, R, Mk, (B)	#4829 (H)	#4486 (H)
#2258 PU.1 (9G7) Rabbit mAb	H, M, (Mk, Pg)		#4486 (H)
#2266 PU.1 Antibody	H, M, (Mk, Pg)		#4486 (H)
#9313 Rb (D20) Rabbit mAb	H, M, Mk	#7001 (H)	#4486 (H)
#9309 Rb (4H1) Mouse mAb	H, Mk, B, Pg	#7001 (H)	#4486 (H)
#2629 Rpb1 CTD (4H8) Mouse mAb	H, M, R, Mk, (Hm, Dm, Sc)	#4471 (H), #5516 (H)	#4490 (H), #4493 (H)
#5694 RING1B (D22F2) XP [®] Rabbit mAb	H, M, R, Mk	#5517 (H)	#4486 (H)
#9511 Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad8 (Ser426/428) Antibody	H, M, R, Mi, X	#5139 (H)	#4486 (H)
#6944 Smad1 (D59F7) XP [®] Rabbit mAb	H, M, (Mk, X, B)	#5139 (H)	#4486 (H)
#9743 Smad1 Antibody	H, M, Mk	#5139 (H)	#4486 (H)
#5339 Smad2 (D43B4) XP [®] Rabbit mAb	H, M, R, Mk	#4669 (H), #5139 (H)	#4486 (H)
#5678 Smad2/3 Antibody	H, M, R, Mk	#4669 (H), #5139 (H)	#4486 (H)
#9520 Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb	H, M, R, (Mk, X, Z, B)	#4669 (H), #5139 (H)	#4486 (H)
#9523 Smad3 (C67H9) Rabbit mAb	H, M, R, Mk, (X, Z, B)	#4669 (H), #5139 (H)	#4486 (H)
#9515 Smad4 Antibody	H, M, R, Mk	#4669 (H), #5139 (H)	#4486 (H)
#5024 Sox2 (D6D9) XP [®] Rabbit mAb (ChIP Formulated)	H, (Mk, B, Dg)	#4641 (H), #4649 (H)	#4486 (H)
#2748 Sox2 Antibody	H, M, (R, Mk, B, Dg)	#4641 (H), #4649 (H), #4653 (M), #4659 (M)	#4486 (H), #7015 (M)
#5147 SRF (D71A9) XP [®] Rabbit mAb	H, M, R, Mk, Pg	#4663 (H)	#4486 (H)
#9167 Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb	H, M	#5148 (H)	#4486 (H)
#9171 Phospho-Stat1 (Tyr701) Antibody	H, M, R, (B, Dg)	#5148 (H)	#4486 (H)
#9177 Phospho-Stat1 (Ser727) Antibody	H, M, R, (B)	#5148 (H)	#4486 (H)
#9172 Stat1 Antibody	H, M, R, Mk, (B, Dg)	#5148 (H)	#4486 (H)
#9145 Phospho-Stat3 (Tyr705) (D3A7) XP [®] Rabbit mAb	H, M, R, Mk	#4663 (H)	#4486 (H)
#9131 Phospho-Stat3 (Tyr705) Antibody	H, M, R, Mk, (C, B)	#4663 (H)	#4486 (H)
#9134 Phospho-Stat3 (Ser727) Antibody	H, M, R, (B)	#4663 (H)	#4486 (H)
#4904 Stat3 (79D7) Rabbit mAb	H, M, R, Mk	#4663 (H)	#4486 (H)
#9139 Stat3 (124H6) Mouse mAb	H, M, R, Mk	#4663 (H)	#4486 (H)
#9132 Stat3 Antibody	H, M, R, (B)	#4663 (H)	#4486 (H)
#5267 Phospho-Stat4 (Tyr693) Antibody	H, (M, R)		#4486 (H)
#2653 Stat4 (C46B10) Rabbit mAb	H, M, R		#4486 (H)
#9351 Phospho-Stat5 (Tyr694) Antibody	H, M, (R, B)	#5131 (M)	#7015 (M)
#9358 Stat5 (3H7) Rabbit mAb	H, M, R	#5131 (M)	#7015 (M)
#9363 Stat5 Antibody	H, M, R	#5131 (M)	#7015 (M)
#3737 SUZ12 (D39F6) XP [®] Rabbit mAb	H, M, R, Mk	#4493 (H)	#4486 (H)
#2569 TCF4 (C48H11) Rabbit mAb	H, (M, R, C)	#5111 (H)	#4486 (H)
#2565 TCF4 (C9B9) Rabbit mAb	H, (M, R)	#5111 (H)	#4486 (H)

Isotype Controls

#5415 Mouse (G3A1) mAb IgG1 Isotype Control			
#2729 Normal Rabbit IgG			

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® Assay Kits

10 assays

New 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

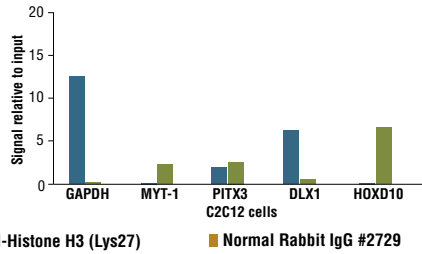
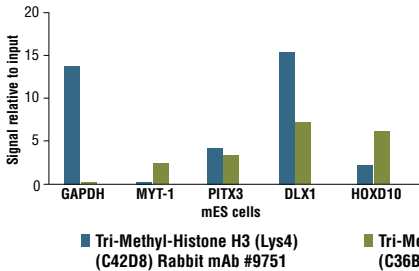
Nanog (D73G4) XP® Rabbit mAb (ChIP Formulated) #5232
Oct-4A (C30A3C1) Rabbit mAb (ChIP Formulated) #5677
Sox2 (D6D9) XP® Rabbit mAb (ChIP Formulated) #5024
SimpleChIP® Human Oct-4 Promoter Primers #4641
SimpleChIP® Human α Satellite Repeat Primers #4486

#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



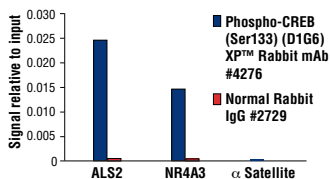
SimpleChIP® Mouse Bivalent Promoter Assay Kit #8981: Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 mES cells (left panel) or C2C12 cells (right panel) and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb, Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb, or 2 μ 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.

XP® Monoclonal Antibodies

eXceptional Performance™

XP® Monoclonal Antibodies are a line of high quality rabbit monoclonal antibodies exclusively available from Cell Signaling Technology. Products labeled with XP have been carefully selected based on superior performance in all approved applications. XP Monoclonal Antibodies are generated using XMT® technology, a proprietary monoclonal method allowing more comprehensive screening and the identification of XP monoclonal antibodies.

eXceptional specificity
+ **eXceptional sensitivity**
+ **eXceptional stability and reproducibility**
= **eXceptional Performance™**



Phospho-CREB (Ser133) (D166) XP® Rabbit mAb #4276: Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 293 cells treated with Forskolin #3828 (30 μ M) for 1 hour and either 10 μ l of Phospho-CREB (Ser133) (D166) XP® Rabbit mAb or 2 μ l of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using human ALS2 exon 1 primers, SimpleChIP® Human NR4A3 Promoter Primers #4829, 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).

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 oligo-nucleotide 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

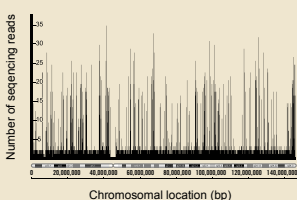


Figure 2B

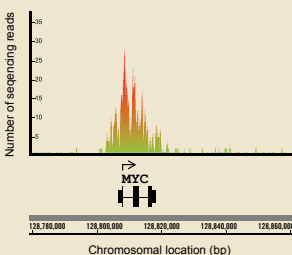
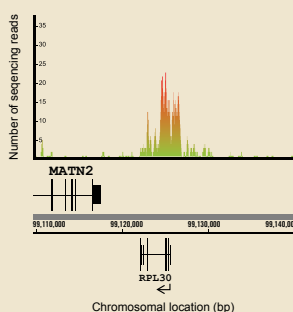


Figure 2C



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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