#8981 Store at -20°C

SimpleChIP® Mouse Bivalent Promoter Assay Kit

☑ 1 Kit
(10 immunoprecipitations)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Application	Dilution
Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb	9751	10 immunoprecipitations	ChIP	1:50
Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb	9733	10 immunoprecipitations	ChIP	1:50
SimpleChIP® Mouse GAPDH Intron 2 Primers	8986	250 PCR reactions	ChIP	1:10
SimpleChIP® Mouse MYT-1 Promoter Primers	8985	250 PCR reactions	ChIP	1:10
SimpleChIP® Mouse PITX3 Intron 1 Primers	8984	250 PCR reactions	ChIP	1:10

Description: The SimpleChIP® Mouse Bivalent Promoter Assay Kit contains ChIP-formulated antibodies and SimpleChIP® primers for the analysis of tri-methyl histone H3 Lys4 and Lys27 marks on target genes in mouse cells by chromatin immunoprecipitation (ChIP). SimpleChIP® Mouse GAPDH Intron 2 Primers are provided as a positive control for enrichment of tri-methyl Lys4, as GAPDH is a housekeeping gene that is heavily enriched for active histone marks. SimpleChIP® Mouse MYT-1 Promoter Primers are provided as a positive control for enrichment of tri-methyl Lys27 enrichment, as MYT-1 is repressed by polycomb proteins in most cell lines. SimpleChIP® Mouse PITX3 Intron 1 Primers are provided for enrichment of both marks, as PITX3 has been shown to be bivalent in many cell types (6). Antibodies and primers are tested and optimized for parallel use with the SimpleChIP® Enzymatic Chromatin IP Kits #9002 and #9003 and SYBR® Green quantitative real-time PCR. The kit provides enough reagents for 10 ChIP assays per antibody and 250 PCR reactions per primer set.

Species Cross-Reactivity: M

Specificity/Sensitivity: Each antibody in the SimpleChIP® Mouse Bivalent Promoter Assay Kit detects endogenous levels of its respective modified histone protein. SimpleChIP® Mouse GAPDH Intron 2 Primers contain a mix of PCR primers that are specific for amplification of a 200 base pair region of the mouse GAPDH gene. SimpleChIP® Mouse MYT-1 Promoter Primers contain a mix of PCR primers that are specific for the amplification of a 211 base pair region of the mouse MYT-1 gene. SimpleChIP® Mouse PITX3 Intron 1 Primers contain a mix of PCR primers that are specific for the amplification of a 248 base pair region of the mouse PITX3 gene.

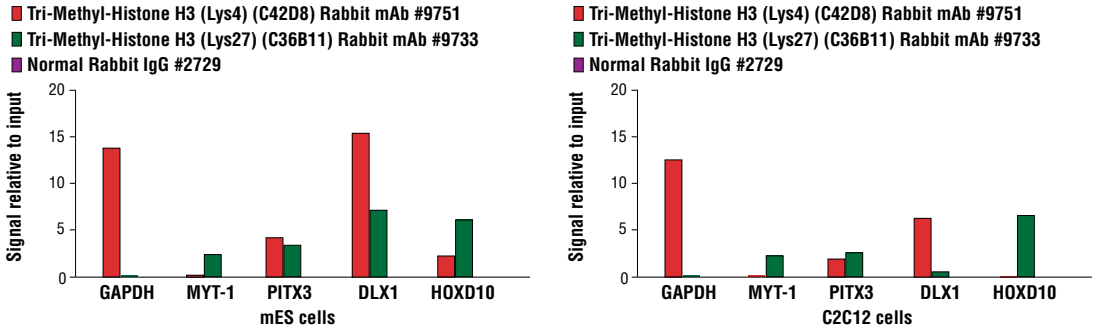
Source/Purification: Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb is produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of histone H3 in which Lys4 is tri-methylated. Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb is produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of histone H3 in which Lys27 is tri-methylated.

Storage: Antibodies are supplied in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide, and should be stored at -20°C. *Do not aliquot the antibodies.*

Primers are supplied in nuclease-free water at a concentration of 5 µM and should be stored at -20°C.

SYBR® Green is a registered trademark of Molecular Probes, Inc.

Background: The nucleosome, made up of four core histone proteins (H2A, H2B, H3 and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have now been shown to be dynamic proteins, undergoing multiple types of post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination (1). Histone methylation is a major determinant for the formation of active and inactive regions of the genome and is crucial for the proper programming of the genome during development (2,3). Trithorax proteins catalyze the tri-methylation of histone H3 Lys4, a mark of transcriptional activation, while polycomb proteins establish and maintain tri-methylation of histone H3 Lys27, a mark of transcriptional repression (4,5). Though originally thought to be mutually exclusive, recent studies have shown that in stem cells certain developmental genes and highly conserved non-coding elements contain both of these marks (6-8). These 'bivalent' regions of the genome are poised for activation and are thought to hold the key to the vast potential of stem cells. As stem cells differentiate along a given lineage, many bivalent genes become monovalent, either retaining the tri-methyl histone H3 Lys4 mark if activated during differentiation, or the tri-methyl-histone H3 Lys27 mark if repressed. Chromatin immunoprecipitation (ChIP) is a powerful technique that can be used to identify bivalent domains in stem cells and changes in bivalency that occur during differentiation (6-8).



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 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 µl 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 differenitated cell line C2C12.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Directions for Use:

A. Chromatin Immunoprecipitation:

ChIP formulated antibodies have been tested and optimized using the SimpleChIP® Enzymatic Chromatin IP Kits (#9002 and #9003). Antibodies should be used at a dilution of 1:50 in a 500 µl ChIP reaction containing 10 to 15 µg of chromatin ($4x10^6$ cells). For the SimpleChIP® Enzymatic Chromatin IP protocol, please see the web page for this product at www.cellsignal.com.

B. Quantification of DNA by qPCR:

1. Label the appropriate number of PCR tubes or PCR plates compatible with the model of real-time PCR machine to be used. PCR reactions should be performed in duplicate and should include a tube with no DNA to control for contamination, and a serial dilution of a 2% total input chromatin DNA (undiluted, 1:5, 1:25, 1:125), which is used to create a standard curve and determine amplification efficiency.

2. Add 2 µl of the appropriate ChIP DNA sample to each tube or well of the PCR plate.

3. Prepare a master PCR reaction mix as described below. Add enough reagents for two extra reactions to account for loss of volume. Add 18 µl of the master PCR reaction mix to each PCR reaction tube or well of the PCR plate.

Reagent	Volume for 1 PCR Reaction (20 µl)
Nuclease-free H_2O	6 µl
5 µM SimpleChIP® Primers	2 µl
2X SYBR® Green Reaction Mix	10 µl

4. Start the following PCR reaction program:
   a. Initial Denaturation: 95°C for 3 min
   b. Denaturation: 95°C for 15 sec
   c. Anneal and Extension 65°C for 60 sec
   d. Repeat steps b and c for a total of 40 cycles.

5. Analyze quantitative PCR results using software provided with the real-time PCR machine.

Background References:

(1) Peterson, C.L. and Laniel, M.A. (2004) *Curr Biol* 14, R546-51.

(2) Kubicek, S. et al. (2006) *Ernst Schering Res Found Workshop* , 1-27.

(3) Lin, W. and Dent, S.Y. (2006) *Curr Opin Genet Dev* 16, 137-42.

(4) Byrd, K.N. and Shearn, A. (2003) *Proc Natl Acad Sci USA* 100, 11535-40.

(5) Cao, R. et al. (2002) *Science* 298, 1039-43.

(6) Bernstein, B.E. et al. (2006) *Cell* 125, 315-26.

(7) Pan, G. et al. (2007) *Cell Stem Cell* 1, 299-312.

(8) Mikkelsen, T.S. et al. (2007) *Nature* 448, 553-60.

MSDS

SimpleChIP™ Primers

I. Identification:

Product name: SimpleChIP™ Primers
Product Catalog: 4471, 4478, 4486, 4490, 4493, 4641, 4649, 4653, 4659, 4663, 4669, 4779, 4829, 5037, 5047, 5077, 5098, 5111, 5131, 5139, 5148, 5156, 5172, 5549, 5550, 5551, 5552, 7014, 7015
CAS#: None
Manufacturer Supplier: Cell Signaling Technology
3 Trask Lane
Danvers, MA 01923 USA
978-867-2300 TEL
978-867-2400 FAX
978-578-6737 EMERGENCY TEL

II. Composition/Information:

This preparation is composed of deoxyribonucleic acid oligonucleotides in water. Considered non-hazardous.
CAS#: N/A

III. Hazard Identification:

CAUTION: This product is not for use in humans. It is intended for research purposes only. To the best of our knowledge, the chemical, physical, and toxicological properties of this material have not been established.

EMERGENCY OVERVIEW OF PRODUCT
OSHA: No known hazards.
This substance is not classified as dangerous according to Directive 67/548/EEC.

IV. First Aid Measures:

Inhalation: If inhaled, remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If swallowed, wash out mouth with water provided person is conscious. Get medical attention.
Skin exposure: In case of contact, immediately wash skin with soap and water for at least 15 minutes. Remove contaminated clothing. Wash clothing before reuse.
Eye exposure: In case of contact with eyes, immediately flush eyes with water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Data not available.
Autoignition Temperature: Data not available.
Explosion: Data not available.
Fire extinguishing media: Water spray, dry chemical, alcohol foam, or carbon dioxide.
Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes. May emit toxic fumes under fire conditions.

VI. Accidental Release Measures: Wear appropriate personal protective equipment. Wash spill site thoroughly.

VII. Handling And Storage:

Store in tightly closed container at -20°C. Avoid inhalation. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling.

VIII. Exposure Controls/Personal:

Ventilation System: A system of local and/or general exhaust is recommended.
Skin Protection: Wear compatible chemical resistant gloves and protective clothing.
Eye protection: Wear protective safety glasses or chemical safety goggles. Maintain eye wash fountain and quick-drench facilities in work area.

IX. Physical And Chemical Properties

Appearance:	colorless liquid
Odor:	odorless
pH:	data not available
Melting Point:	data not available
Boiling Point:	data not available
Freezing Point:	data not available
Volatile Organic Compounds:	data not available
Solubility in water:	soluble in water

X. Stability and Reactivity:

Stability: Stable under normal conditions.
Conditions/materials to avoid: Data not available.
Hazardous Decomposition: Data not available.

XI. Toxicological Information:

Acute Effects: Not established.
Chronic Effects: Not established.
Potential Health Effects: Not established.
Inhalation: May be harmful if inhaled.
Skin: May be harmful if absorbed through skin.
Eyes: Causes eye irritation.
Ingestion: May be harmful if swallowed.

XII. Ecological Information: No data available.

XIII. Disposal Considerations: Dispose of in accordance with federal, state, local environmental regulations.

XIV. Transport Information:

DOT: Not dangerous goods.
ADR/RID: Not dangerous goods.
IMDG: Not dangerous goods.
IATA : Not dangerous goods.

XV. Regulatory Information:

EU Regulations/Classifications/Labeling Information: None.
US Regulatory Information:
SARA Listed: No.
Canada (WHMIS): DSL No, NDSL No.

XVI. Other Information:

This compound is sold only for research use only. It is not for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.

Material Safety Data Sheet (MSDS) for Antibodies

rev. 06/02/09

I. Identification:

Product name: Antibodies
Product Catalog Number: Includes antibodies within the following range of catalog numbers: 2000-5999, 7000-7999 and 9000-9999.
CAS number: None

Manufacturer Supplier: Cell Signaling Technology
3 Trask Lane
Danvers, MA 01923 USA
1-978-867-2300 TEL
1-978-867-2400 FAX
1-978-578-6737 Emergency Phone

II. Composition/Information on Ingredients:

This product is composed of antibodies in aqueous buffer solution. According to 29 CFR 1910.1200(d), hazardous ingredients at less than <1% and carcinogens at less than < 0.1% are considered non-hazardous. Any hazardous or carcinogenic ingredients exceeding these criteria are listed below.

This product may contain the following hazardous ingredients.

III. Hazard Identification:

Emergency Overview of Hazardous ingredient: Glycerol (CAS# 56-81-5)
Caution: Avoid contact and inhalation.
Target Organ: Kidneys.

Ingredient	CAS#	Percent
Glycerol	56-81-5	50%

NFPA Rating:

Health Rating:	1
Flammability Rating:	0
Reactivity Rating:	0

IV. First Aid Measures:

Inhalation: If inhaled, remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If swallowed and person is conscious, rinse out mouth with water. Get medical attention.
Skin Exposure: In case of contact, wash skin with soap and water.
Eye Exposure: In case of contact with eyes, immediately flush eyes water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Data not available.
Autoignition Temperature: Data not available.
Fire Extinguishing Media: Water spray, dry chemical, foam, or carbon dioxide.
Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.

VI. Accidental Release Measures:

Absorb liquid with an absorbent material. Transfer contaminated absorbent to a chemical waste container for disposal.

VII. Handling And Storage:

Avoid inhalation and contact with eyes and skin. Avoid prolonged or repeated exposure. Store at –20°C in tightly closed container.

VIII. Exposure Controls/Personal

Engineering Controls: Maintain adequate ventilation, eye wash and quick-drench facilities in work area.
Personal Protective Equipment: Lab coat, chemical resistant gloves and chemical safety glasses.
Occupational Exposure Limits: Data not available.

IX. Exposure Controls/Personal Protection:

Physical State:	Colorless liquid.
Odor:	Odorless.
Boiling Point:	Data not available.
Melting Point:	Data not available.
Volatile Organic Compound:	Data not available.
Solubility in water:	Readily miscible in water.

X. Stability and Reactivity:

Stability: Stable.
Hazardous Decomposition: May form carbon dioxide and carbon monoxide.
Conditions to avoid: Strong oxidizing agents

XI. Toxicological Information:

May cause skin irritation.
May be toxic if absorbed through skin or ingested.
May cause eye irritation.

Target Organs: Kidneys
Prolonged exposure may cause nausea, headache, and vomiting.

XII. Ecological Information:

Data not available.

XIII. Disposal Considerations:

Dispose of in accordance with federal, state and local environmental regulations.

XIV. Transport Information:

D.O.T.: This substance is considered non-hazardous for transport.
IATA: This substance is considered non-hazardous for air transport.

XV. Regulatory Information:

EU Regulation/Classification/Labeling Information: Not available for this product.

Chemical Inventory Status:
SARA Listed Component: None.
TSCA Listed Component: None.
Canada (WHMIS): DSL No, NDSL No.

XVI. Other Information:

This compound is sold only for research use by personnel familiar with chemicals and who are well trained in good laboratory habits, such as avoiding spills, keeping hands clean at all times and not rubbing eyes with hands while working in the laboratory.

This solution is sold only in microliter quantities for use in life sciences research. No other use is intended, and any other use may involve substantive hazards.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide for experienced personnel. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product. The burden of safe use of this material rests entirely with the user.