

SimpleChIP™ Stem Cell Master Regulator Assay Kit

✓ 1 Kit
(10 immunoprecipitations)



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

Products Included	Product #	Quantity	Application	Dilution
Oct-4A (C30A3C1) Rabbit mAb (ChIP Formulated)	5677	10 immunoprecipitations	ChIP	1:50
Sox2 (D6D9) XP™ Rabbit mAb (ChIP Formulated)	5024	10 immunoprecipitations	ChIP	1:50
Nanog (D73G4) XP™ Rabbit mAb (ChIP Formulated)	5232	10 immunoprecipitations	ChIP	1:50
SimpleChIP™ Human Oct-4 Promoter Primers	4641	250 PCR reactions	ChIP	1:10
SimpleChIP™ Human α Satellite Repeat Primers	4486	250 PCR reactions	ChIP	1:10

Storage: Antibodies are supplied in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 μ g/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 μ M and should be stored at -20°C.

Background: Embryonic stem cells are pluripotent cells derived from the inner cell mass (ICM) of the mammalian blastocyst. Pluripotent cells are capable of indefinite self-renewing expansion in culture and can differentiate into cell types of all three germ layers: endoderm, ectoderm and mesoderm. This pluripotent state is a property shared by embryonic stem (ES) cells, embryonic carcinoma and induced pluripotent stem (iPS) cells. Oct-4, Sox2 and Nanog are key transcriptional regulators that are highly expressed in pluripotent cells (1). Together they form a master transcriptional regulatory network that maintains cells in a pluripotent state (2,3). Over-expression of Oct-4 and Sox2 along with Klf4 and c-Myc can induce pluripotency in both mouse and human somatic cells, highlighting their roles as key regulators of the transcriptional network necessary for self-renewal and pluripotency (4,5). It has also been demonstrated that overexpression of Oct-4, Sox2, Nanog and Lin28 can induce pluripotency in human somatic cells (6). Chromatin immunoprecipitation (ChIP) is a powerful technique that can be used to identify Oct-4, Sox2 and Nanog target genes in a given population of pluripotent cells (2,3,7-9). In addition, ChIP can be used to characterize changes in target gene occupancy that occur during induction of iPS cells from somatic cells, or differentiation of pluripotent cells into different cell lineages.

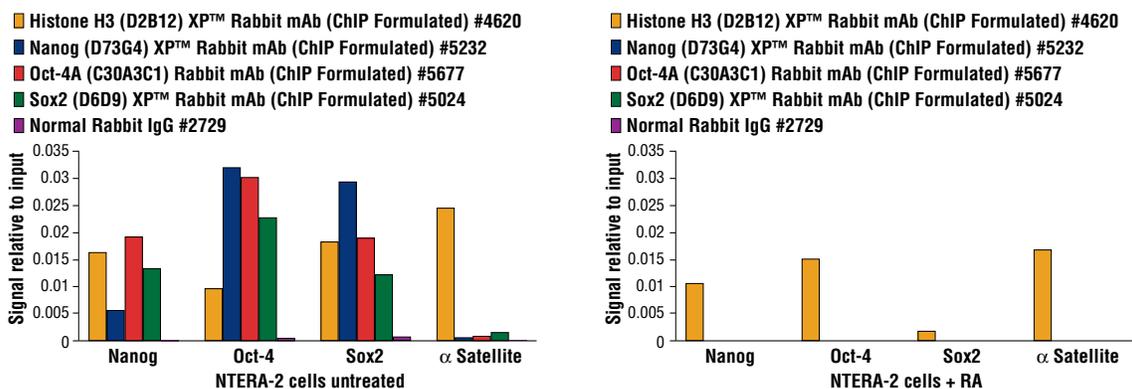
SYBR® Green is a registered trademark of Molecular Probes, Inc.

Description: The SimpleChIP™ Stem Cell Master Regulator Assay Kit contains ChIP-formulated antibodies and SimpleChIP™ primers for the analysis of Oct-4, Sox2 and Nanog binding to target genes in human cells by chromatin immunoprecipitation (ChIP). The positive control SimpleChIP™ Human Oct-4 Promoter Primers are provided for detection and quantification of Oct-4 promoter enrichment, as Oct-4 is a known target gene of Oct-4, Sox2 and Nanog proteins. The negative control SimpleChIP™ Human α Satellite Repeat Primers allow for determination of background levels of enrichment. 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.

Specificity/Sensitivity: Each antibody in the SimpleChIP™ Stem Cell Master Regulator Assay Kit detects endogenous levels of its respective human protein. SimpleChIP™ Human Oct-4 Promoter Primers contain a mix of forward and reverse PCR primers that are specific for amplification of a 99 base pair region of the human Oct-4 promoter. SimpleChIP™ Human α Satellite Repeat Primers contain a mix of forward and reverse PCR primers that are specific for the amplification of a 182 base pair region of the human α satellite repeat element.

Source/Purification: Oct-4 (C30A3C1) Rabbit mAb (ChIP Formulated) is produced by immunizing animals with recombinant protein specific to the amino terminus of human Oct-4A protein. Sox2 (D6D9) XP™ Rabbit mAb (ChIP Formulated) is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly179 of human Sox2 protein. Nanog (D73G4) XP™ Rabbit mAb (ChIP Formulated) is produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of human Nanog protein.

Species Cross-Reactivity: H



NTERA-2 cells were either untreated (left panel) or treated for 15 days with retinoic acid (RA) to induce differentiation along the neuronal lineage (right panel). Chromatin immunoprecipitations were then performed with cross-linked chromatin from 4×10^6 cells and 10 μ l of Nanog, Oct-4, and Sox2 antibodies, 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 human Nanog promoter primers, SimpleChIP™ Human Oct-4 Promoter Primers #4641, SimpleChIP™ Human Sox2 Promoter Primers #4649, 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, which is equivalent to one. Note the loss of Nanog, Oct-4, and Sox2 binding to target genes as NTERA-2 cells are induced to differentiate.

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 (4×10^6 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 ₂ O	6 µl
5 µM SimpleChIP™ Primers	2 µl
2X SYBR® Green Reaction Mix	10 µl

4. Start the following PCR reaction program:

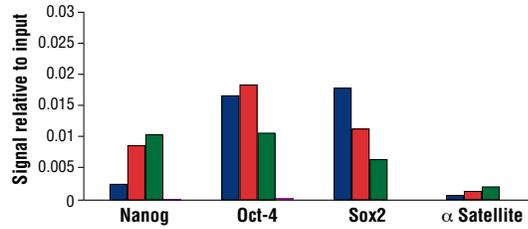
- Initial Denaturation: 95°C for 3 min
- Denaturation: 95°C for 15 sec
- Anneal and Extension: Primer-specific temp. for 60 sec
- Repeat steps b and c for a total of 40 cycles.

*65 °C Anneal/Extension for SimpleChIP™ Human Oct-4 Promoter Primers

*60 °C Anneal/Extension for SimpleChIP™ Human α Satellite Repeat Primers

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

- Nanog (D73G4) XP® Rabbit mAb (ChIP Formulated) #5232
- Oct-4A (C30A3C1) Rabbit mAb (ChIP Formulated) #5677
- Sox2 (D6D9) XP® Rabbit mAb (ChIP Formulated) #5024
- Normal Rabbit IgG #2729



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 NCCIT cells and 10 µl of Nanog, Oct-4 and Sox2 antibodies, 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 human Nanog promoter primers, SimpleChIP™ Human Oct-4 Promoter Primers #4641, SimpleChIP™ Human Sox2 Promoter Primers #4649, 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, which is equivalent to one.

Background References:

- (1) Looijenga, L.H. et al. (2003) *Cancer Res* 63, 2244-50.
- (2) Boyer, L.A. et al. (2005) *Cell* 122, 947-56.
- (3) Loh, Y.H. et al. (2006) *Nat Genet* 38, 431-40.
- (4) Takahashi, K. and Yamanaka, S. (2006) *Cell* 126, 663-76.
- (5) Okita, K. et al. (2007) *Nature* 448, 313-7.
- (6) Yu, J. et al. (2007) *Science* 318, 1917-20.
- (7) Okumura-Nakanishi, S. et al. (2005) *J Biol Chem* 280, 5307-17.
- (8) Catena, R. et al. (2004) *J Biol Chem* 279, 41846-57.
- (9) Rodda, D.J. et al. (2005) *J Biol Chem* 280, 24731-7.

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, 5516, 5517, 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.