Magna ChIP™ HiSens

Catalog No. 17-10460

EZ-Magna ChIP™ HiSens

Catalog No. 17-10461

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

USA & Canada Phone: +1(800) 645-5476
In Europe, please contact Customer Service:
France: 0825.045.645;
Spain: 901.516.645 Option 1
Germany: 01805.045.645
Italy: 848.845.645
United Kingdom: 0870.900.46.45
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Introduction

DNA in eukaryotic cells is associated with a plethora of structural, enzymatic, and regulatory proteins which may interact with regional DNA sequences and affect genomic functions via packaging as chromatin. This dynamic and coordinated interaction may influence the expression and cellular utilization of each gene locus, whether coding or non-coding. Additionally, such proteins and in some cases, non-coding RNAs influence genomic DNA's ability to replicate and repair itself in a cell cycle dependent manner. Thus, it is crucial to elucidate these DNA-protein interactions in order to decipher the nuclear mechanisms underlying a wide variety of biological processes and disease states.

ChIP (chromatin immunoprecipitation) is a powerful technique classically used for mapping the in vivo distribution of proteins associated with chromosomal DNA. These proteins can be histone subunits, transcription factors, or other regulatory or structural proteins bound either directly or indirectly to DNA. Successful ChIP requires high quality ChIP-validated antibodies that can specifically detect proteins associated with regions of chromosomal DNA. Traditionally, endpoint or quantitative PCR (qPCR) is performed after ChIP to verify whether a particular DNA sequence (the gene or region of the genome) is associated with the protein of interest. Using this classical approach, researchers can evaluate the interactions of the proteins of interest with a limited number of known target genes.

Alternatively ChIP can be used to map protein-DNA interactions on a genome wide basis to produce epigenomic maps. This can be done for multiple marks at a given locus as well as across the genome. The need to profile these interactions across the genome has led to the development of genome-wide ChIP analyses with either microarrays (ChIP-chip) or next-generation sequencing (ChIP-seq)†. Genome-wide mapping of protein-DNA interactions and epigenetic marks helps to elucidate mechanisms of transcriptional control, and maintenance of chromatic and genomic DNA integrity within a cell. In certain cases the profile of this epigenome can be used to assess progression through differentiation; distinguish between normal and disease states; or predict responses to inflammation or pharmacologic treatment.

Both high and low abundance chromatin associated proteins are amenable to ChIP analysis. However, this requires reagents that work reliably with a wide range of input chromatin amounts while still providing high levels of enrichment and low background signals. The Magna ChIP HiSens kit has been designed to work well for both high and low abundance proteins and a wide range of input amounts of chromatin. Unlike other kits used for only low abundance targets or limited amounts of chromatin, the Magna ChIP HiSens kit is a universal kit that can eliminate the need to have multiple types of kits for different targets, input chromatin amounts, and antibody isotypes. This extremely sensitive ChIP kit is able to achieve this flexibility through the use of a specialized blend of magnetic protein A/G beads and a unique, proprietary multi-purpose buffer system. Compatible with a variety of downstream analysis approaches such as qPCR and next generation sequencing (ChIP-seq), the design of this kit enables superior enrichment of low and high abundance targets from as few as 10,000 to as many as 1,000,000 cells with extremely low IgG backgrounds resulting in very high signal to noise ratios.

† The Magna HiSens kit is compatible with downstream analysis using next generation sequencing. For information on creating next generation sequencing libraries for genome-wide analysis, please refer to the Magna ChIP-Seq™ Chromatin Immunoprecipitation and Next Generation Sequencing Library Preparation Kit user manual (Cat. No. 17-1010).

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Kit Overview

Description

The Magna ChIP HiSens and EZ-Magna ChIP HiSens kits provide a complete set of validated, quality controlled reagents, and a detailed protocol to enable ChIP from a wide range of input amounts of chromatin obtained from either cells or tissues. Our specialized blend of protein A/G blend of magnetic beads is specifically produced for chromatin immunoprecipitation and enables the use of a broader range of antibodies than protein A or G alone eliminating the need to purchase different kits for different antibody isotypes. The SCW Buffer is unique to the Magna ChIP HiSens kit and enables the use of a single buffer for multiple steps of the ChIP process (sonication, chromatin immunoprecipitation, and wash). The ChIP elution buffer provided with the HiSens kit has been formulated to allow analysis of enrichment by qPCR without additional clean-up steps for more rapid results.

The materials provided allow twelve chromatin preparations and up to 24 ChIP assays. The Magna ChIP HiSens kit is designed to work well with a range of input amounts of chromatin. Using the reagents and protocols provided with this kit, ChIP reactions can be performed with as few as 10,000 cell equivalents of chromatin when exploring abundant proteins such as histones and common transcription factors, and as few as 100,000 cell equivalents when measuring less abundant proteins such as sequence-specific transcription factors. If necessary for very rare factors, chromatin equivalents and bead quantities per ChIP can be scaled up or multiple reactions can be run and pooled to obtain sufficient DNA for ChIP-seq experiments. Please refer to “Chromatin Immunoprecipitation: Experimental Considerations” section on pages 9-10 and protocol for specific guidance.

Differences Between EZ-Magna ChIP HiSens Kit and Magna ChIP HiSens Kits

Just like the Magna ChIP HiSens kit, the EZ-Magna ChIP HiSens kit contains all of the materials required for ChIP. However in addition to these materials, the EZ-Magna ChIP version of this kit includes positive and negative control antibodies and PCR primers. These reagents can be used as in-process controls, or for verification of technique.

Magna ChIP HiSens Kits Performance Characteristics

Features and Advantages

- Robust ChIP from as few as 10,000 to as many as 1,000,000 cells
- Protocols and reagents for generation of chromatin from range of input sample type
- Superior performance with variety of isotypes of either polyclonal or monoclonal antibodies
- Specialized buffer and bead formulations; lower backgrounds and higher fold enrichment
- Single buffer system for sonication, chromatin IP, and wash
- Perform analysis of enrichment without additional purification after cross link reversal.
- Compatible with all commonly used downstream analysis applications– qPCR, next generation sequencing, microarray

Magnetic A/G Blend – Better Signal to Noise Ratios from Wider Range of Antibodies

Magna ChIP HiSens kit and EZ-Magna ChIP HiSens kits utilize a magnetic protein A/G bead blend that provides multiple advantages over both agarose beads and single-magnetic bead approaches. In contrast to kits with either protein A or protein G, our Magna ChIP A/G beads allow use of a wider variety of antibody isotypes. In addition, the magnetic properties of these beads permit rapid magnetic processing of ChIP reactions using either manual or automated methods.

Optimized Buffer System and Kit Design: Easier Protocol and Improved Signal to Noise Ratios

The Magna ChIP HiSens kits use a streamlined protocol and a single buffer for sonication, ChIP and washing steps. This approach makes the kits easier to use and makes the protocol more easily adapted to automated platforms.

The Magna ChIP HiSens kits are designed and optimized for efficient immunoprecipitation and recovery of DNA with reduction of the amount of chromatin required per ChIP. The new simplified washing procedure and magnetic bead protein A/G blend work synergistically resulting in significant improvements in signal to noise ratios.

As a result of this improved procedure, DNA purification is not required after cross link reversal prior to qPCR analysis of immunoprecipitated material. This is especially true when using the Magna ChIP kit with high quality antibodies to abundant epitopes such as histone modifications and nuclear enzymes. However, to concentrate low abundance targets or for other downstream analysis application such as ChIP-seq, researchers may consider the use of DNA purification products such as the Agencourt® AMPure® XP DNA Clean beads or other small-volume elution DNA purification products to concentrate the final DNA product prior to qPCR detection, construction of next generation sequencing libraries, or labeling isolated DNA fragments for microarray analysis. To make it easier to perform these steps, an optional protocol for the use of Agencourt beads (not supplied) in combination with the Magna ChIP HiSens kit is provided.

Kit Components

The Magna ChIP HiSens kit provides sufficient reagents for 12 individual chromatin preparations and 24 chromatin immunoprecipitations. The EZ-Magna ChIP HiSens kit includes these reagents plus positive and negative control antibodies and a human genome specific primer set for qPCR analysis. Please refer to the table below for details on kit components

Magna ChIP HiSens **Chromatin Immunoprecipitation Kit Contents** **Kit Configurations**	
Magna ChIP HiSens **(Cat. No. 17-10460)**	**EZ-Magna ChIP HiSens** **(Cat. No. 17-10461)**
MAGNA0025 (2°C to 8°C)	**MAGNA0025 (2°C to 8°C)**
MAGNA0023 (-20°C)	**MAGNA0024 (-20°C)**

MAGNA0025 (2°C to 8°C)		
Component	**Item #**	**Quantity**
10X Glycine	CS207370	27 mL
Nuclei Isolation Buffer	CS207355	6 mL
10X PBS	CS207371	26 mL
SCW Buffer (Sonication/ChIP/Wash)	CS207372	64 mL
Magna ChIP Protein A/G Magnetic Beads	CS207374	250 µL
Low Stringency IP Wash Buffer	CS207373	26 mL
ChIP Elution Buffer	CS207377	3.6 mL
MAGNA0023 (-20°C) **Store at -20°C Upon Receipt**		
Component	**Item #**	**Quantity**
Protease Inhibitor Cocktail III, Animal Free ****Contains DMSO**	535140-1ML	1.0 mL
Proteinase K Solution, 600mAU/mL	CS207286	0.2 mL
MAGNA0024 (-20°C) **Store at -20°C Upon Receipt**		
Component	**Item #**	**Quantity**
Protease Inhibitor Cocktail III, Animal Free ****Contains DMSO**	535140-1ML	1.0 mL
Proteinase K Solution, 600mAU/mL	CS207286	0.2 mL
Anti-Trimethyl-Histone H3 (Lys4)	CS200580	75 µL
Normal Rabbit IgG	CS200581	75 µL
Control primers	22-004	75 µL

Functional Validation: Magna ChIP HiSens modules are functionally tested in quantitative ChIP reactions to ensure quality control of the supplied components.

Materials Required But Not Supplied

Reagents

- Cells, stimulated or treated as desired
- Antibody of interest for chromatin immunoprecipitation (see page 6)
- 37% Formaldehyde
- qPCR reagents *(e.g.*SYBR® Green Master Mix
- 2% Agarose gel
- 100 bp DNA Ladder
- 6X gel Loading buffer
- RNAse A 10 mg/ml (e.g. Millipore 70856-3)
- Phenol
- Chloroform:isoamyl alcohol
- Phase Lock tubes
- PCR clean up columns, if organic extraction not desired
- DNase and RNase-free sterile H_2O (e.g. Millipore Nuclease-free water Cat. No. 3098)

Reagents if Performing Next Generation Sequencing Analysis:

- Agencourt® AMPure® XP DNA Clean Beads (Beckman Coulter A63880) or PCR DNA cleanup spin columns
- NGS Library Construction Kit (e.g. Nugen Ovation® Ultralow Library Systems (0303)
- Glycogen

Equipment

- Microscope and cell counter
- Magnetic separation stand (e.g.Magna GrIP™ Rack (8 Well), Cat. No. 20-400)
- Vortex mixer
- Rotating wheel/platform (e.g. Labnet H5500)
- Microcentrifuge
- Sonicator
- Thermomixer® (e.g. Eppendorf 05-400-205)
- Variable temperature water bath or incubator
- Timer
- Variable volume (5-1000 μL) pipettors and tips
- Cell scraper
- Microcentrifuge tubes, 1.5 mL (for best results use siliconized tubes)
- Thermal cycler
- Real-time PCR Instrument
- Filter-tip pipette tips
- Agarose gel electrophoresis equipment or microfluidic electrophoresis system

Hazards

- Wear gloves when using this product. Avoid skin contact or ingestion of all reagents and chemicals used in this protocol.
- Protease Inhibitor Cocktail III contains DMSO, avoid contact with skin.
- Chromatin preparation may require use of liquid nitrogen. Use personal protective equipment (PPE) when handling liquid N_2 to avoid burns.
- Use PPE, fume hoods and venting when working with concentrated formaldehyde solutions. Formaldehyde is toxic by inhalation, skin contact and ingestion.

Storage and Stability

MAGNA0025: Store at 2°C-8ºC; performance guaranteed for 6 months from date of receipt when reagents are stored properly.

MAGNA0023 and MAGNA0024: Store at -20ºC; performance for 6 months from date of receipt when reagents are stored properly.

ChIPAb+™ Validated Antibodies and ChIP Qualified Antibodies

For the ChIP application, not all antibodies are capable of effectively precipitating chromatin. Protein conformation, protein interactions (with other proteins or DNA), and the amount of cross linking can affect whether or not an antibody will work well in ChIP. Consequently, we make a distinction between what we call "ChIP qualified" antibodies, and "ChIP validated" antibodies.

ChIP qualified, or ChIP grade is a term typically used to describe any antibody previously demonstrated to work in ChIP. Although not always directly tested by the supplier, many consider these to be 'validated' for ChIP. For some, this level of validation is sufficient. However, antibody performance in ChIP can vary between different lots. Consequently, in many cases antibodies labeled as ChIP grade fail to perform consistently from lot to lot.

To eliminate this concern, when performing ChIP it is suggested that labs use well characterized antibodies that have been extensively evaluated for specificity, proven to perform in ChIP, and lot validated using ChIP. An example of these types of antibodies is the ChIPAb+ Validated Antibody and Primer Sets. ChIPAb+ antibodies are rigorously validated to ensure specificity and their ability to immunoprecipitate chromatin. In addition, each and every lot of a ChIPAb+ antibody is subject to extensive quality control testing including testing in the ChIP application. ChIPAb+ antibodies are more than just a highly validated antibody. To allow independent verification of performance or for use as a positive control, all ChIPAb+ antibodies include a negative control IgG plus PCR primers directed against a known positive locus. A partial list of ChIPAb+ antibodies is given in the table below.

Catalog Number	Description
17-622	ChIPAb+ Trimethyl-Histone H3 (Lys27)
17-614	ChIPAb+ Trimethyl-Histone H3 (Lys4)
17-658	ChIPAb+ Acetyl-Histone H3 (Lys9)
17-625	ChIPAb+ Trimethyl-Histone H3 (Lys9)
17-648	ChIPAb+ Dimethyl-Histone H3 (Lys9)
17-601	ChIPAb+ Sp1
17-662	ChIPAb+ EZH2, clone AC22
17-615	ChIPAb+ Acetyl Histone H3
17-663	ChIPAb+ EED
17-678	ChIPAb+ Trimethyl-Histone H3 (Lys4)
17-677	ChIPAb+ Dimethyl-Histone H3 (Lys4)
17-10051	ChIPAb+ Acetyl-Histone H3 (Lys14)
17-10050	ChIPAb+ Acetyl-Histone H3 (Lys4)
17-641	ChIPAb+ REST
17-672	ChIPAb+ RNA Pol II
17-608	ChIPAb+ HDAC1
17-10032	ChIPAb+ Trimethyl-Histone H3 (Lys36)
17-661	ChIPAb+ SUZ12
17-10046	ChIPAb+ Histone H3 (C-term)

Catalog Number	Description
17-10044	ChIPAb+ CTCF
17-681	ChIPAb+ Dimethyl-Histone H3 (Lys9)
17-630	ChIPAb+ Acetyl Histone H4
17-613	ChIPAb+ p53
17-603	ChIPAb+ ERα
17-643	ChIPAb+ Monomethyl Histone H3 (Lys27)
17-10048	ChIPAb+ Histone H2A.Z
17-10054	ChIPAb+ Histone H2B
17-10057	ChIPAb+ SMRT
17-10045	ChIPAb+ Acetyl-Histone H4 (Lys5)
17-10098	ChIPAb+ TATA Binding Protein (TBP)
17-10131	ChIPAb+ Phospho-CREB (Ser133)
17-675	ChIPAb+ Histone H3 (Unmod Lys4)
17-10130	ChIPAb+ Trimethyl-Histone H3 (Lys79)
17-600	ChIPAb+ CREB
17-656	ChIPAb+ Sox-2, clone 6F1.2
17-10034	ChIPAb+ EED (Rabbit Poly)
17-685	ChIPAb+ Phospho-Histone H3 (Ser10)
17-620	ChIPAb+ RNA Polymerase II

For a complete listing of Millipore's ChIPAb+ validated antibody/primer sets, visit www.millipore.com/epigenetics and search ChIPAb+
To see all available antibodies visit www.millipore.com/antibodies

Getting the Best Possible Results Using the Magna ChIP™ HiSens Kit

The Magna ChIP HiSens reagents and protocols used in this manual are modified versions of those used in our first generation Magna ChIP kits (catalogue numbers 17-408, 17-409, 17-610, 17-611, 17-10085, 17-10086). The materials provided in the Magna ChIP HiSens kits have been designed for efficient ChIP using lower amounts of input chromatin and antibody. The immunoprecipitation step uses a magnetic protein A/G bead blend to enable lower background signals and the use of a wider range of antibody isotypes than protein A or G alone. In addition, this kit incorporates a streamlined wash procedure to simplify the protocol as well as a proprietary elution buffer that allows the direct analysis of samples by qPCR after cross link reversal without additional clean-up steps.

Similar to our first generation Magna ChIP Chromatin Immunoprecipitation kits, it is possible to perform the ChIP portion of this protocol in a single day using a shortened protocol that reduces incubation time. However, for some antibodies this shorter protocol can result in slightly reduced ChIP efficiency.

Regardless of your approach, to ensure the best possible results, advance planning is advised. It is strongly recommended that you read the entire protocol before performing this procedure, especially if you are using this kit for the first time. A general overview of the major steps of a typical Magna ChIP HiSens workflow is provided on page 11.

The detailed protocols and guidelines presented in this user manual will help you to avoid common pitfalls. It is critical to review and follow the suggestions in the “Experimental Considerations” section. It is also important to evaluate the samples being prepared after key steps in the protocol. This will minimize the potential for sub-optimal results and simplify troubleshooting should problems occur.

Chromatin Immunoprecipitation: Experimental Considerations

ChIP-validated antibodies are perhaps the most important component of a ChIP experiment. Ideally, the antibody being used will have been validated in ChIP using genomic locations of both predicted high and low occupancy (positive vs. negative locus). In addition, verify that the quality of the chromatin being prepared is suitable by evaluating fragmentation by agarose gel electrophoresis and by functional ChIP assays, using control antibodies when possible. If you wish to check your technique or require a source of controls, you can utilize the validated positive and negative control antibodies and qPCR assay provided in the EZ-Magna ChIP HiSens kit or alternatively use a ChIPAb+ validated antibody/primer set (see page 7).

Cell number equivalents of chromatin required per ChIP reaction is dependent on the quantity of available epitopes in the cell of interest as well as the quality of antibody used. Successful enrichment can be performed using the Magna ChIP HiSens kits with as few as $1X10^4$ cells per ChIP when using high affinity antibodies directed towards more abundant proteins such as histone modifications and RNA Polymerase II. If the targets are less abundant or if lower quality or affinity antibodies are used, it is recommended you increase the number of cells.

Chromatin quality is important to the success of ChIP. Chromatin preparation in batch is recommended whenever possible to allow for analysis of fragmentation and greater consistency between experiments. The Magna ChIP HiSens chromatin preparation method used in this manual recommends batch preparation of chromatin. The standard protocol is designed to yield enough chromatin for approximately 10 ChIP reactions per preparation from 1,000,000 cultured cells; or 50 mg of a tissue sample. Enough buffer is provided with this kit to enable chromatin preparation for up to 12 samples (good for 120 reactions at 100,000 cells/ChIP). Should you need to optimize chromatin preparation conditions or if additional chromatin preparation reagents are required the PureEpi™ Chromatin Preparation and Optimization Kit can be used (catalogue # 17-10082). This accessory kit has detailed guidelines for the optimization of chromatin preparation for ChIP and uses the same set of reagents used in the Magna ChIP HiSens kit.

The HiSens protocol works best when the chromatin size is between 200-1000 bp. Shearing of chromatin varies greatly, depending on cell type, growth conditions, quantity of chromatin, volumes used, cross linking conditions, and sonication equipment. It may be necessary to optimize sonication conditions by changing the power settings, cycle number and ratios of time ON and time OFF. The quality of the chromatin should be evaluated by agarose gel electrophoresis of RNase A and Proteinase K-digested, crosslink-reversed purified DNA fragments. An agarose gel or a microfluidic electrophoresis system such as the Agilent BioAnalyzer or similar system can be used to provide a more detailed analysis of the fragmentation of chromatin.

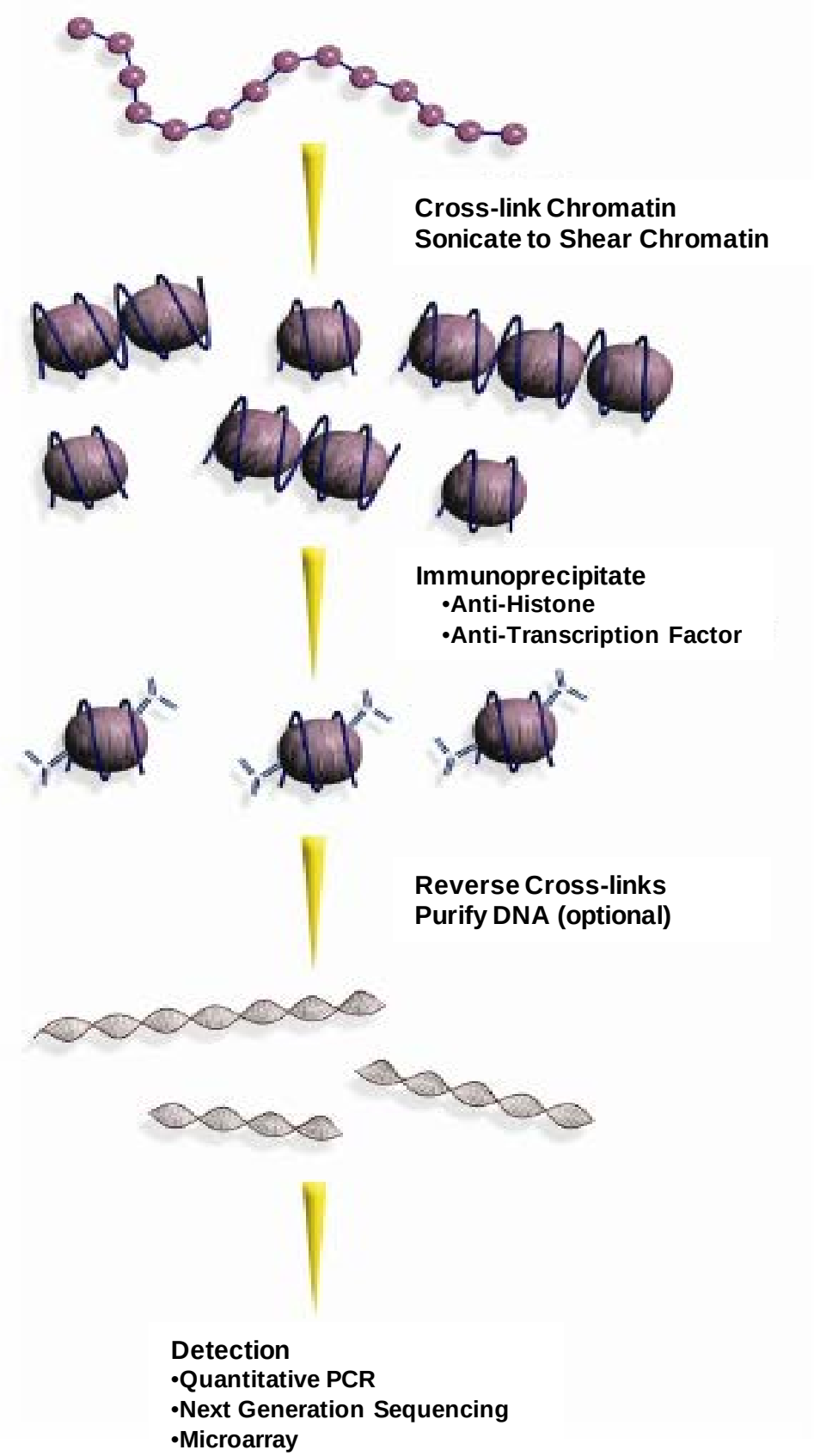
qPCR analysis is typically used to evaluate the success of ChIP. A mock IgG or negative antibody control ChIP reaction may be performed to determine fold enrichment relative to a specific ChIP antibody. Alternatively, a negative locus control may also be used for normalization to minimize chromatin and ChIP reagent requirements.

NGS analysis is a popular detection approach that allows genome wide assessment of protein:DNA binding profiles. When using Magna ChIP HiSens for the purposes of an NGS experiment, it is recommended that more chromatin equivalents be utilized (i.e.1-5e7 cell equivalents per ChIP) and

that the quantity of beads per immunoprecipitation be increased to a minimum of 20 uL per ChIP. Additional Magna ChIP™ A/G beads in 200 μL (catalogue number 16-663X) and 1 mL amounts (16-663) are available should additional bead be required.

In addition when performing ChIP-seq it is critical to carefully monitor the fractionation of your chromatin to ensure that the optimal size range of 200-600 bp is produced. Magna ChIP HiSens is effective at enriching DNA fragments across a broad range of sizes, but NGS library construction requires a narrower range of DNA fragment sizes, so longer sonication times may be required (see section H and Appendix A for details).

Overview of Magna ChIP HiSens Workflow



Protocol

A. Chromatin Immunoprecipitation–Please Read Entire Protocol First

The protocol below covers required steps to produce chromatin for a ChIP experiment. For a more detailed explanation of the variables that affect chromatin quality please consult the manual provided with the PureEpi Chromatin Preparation and Optimization Kit (Cat # 17-10082).
The HiSens kit was optimized for use of chemically cross linked, sonicated chromatin. However, enzymatic protocols may be employed with adjustments to this protocol for using higher cell numbers, starting with Section E. For chromatin preparation using enzymatic digestion instead of mechanical fragmentation, see EZ-Zyme Chromatin Preparation kit (17-375).

B. In Vivo Cross linking of Proteins to DNA

I. Cultured cells (Adherent or Suspension)

1. Prepare cells from chromatin isolation. If necessary, stimulate or treat adherent mammalian cells at ~80 to 90% confluence in a 150 mm culture dish containing 20 mL of growth media. Include one extra plate of cells to be used solely for estimation of cell number.
    - *For HeLa cells, this is approximately 1 x 10^7 cells. This typically generates a preparation of chromatin sufficient for 100 separate immunoprecipitations using 1 X 10^5 cell equivalents/reaction. Use the same amount of buffer when making chromatin from fewer cells.*
    - *The volume of buffers supplied in the kit is sufficient to generate chromatin from up to 12 150 mm plates of cultured cells.*
    - *Cell numbers can be scaled according to the performance of the antibody of interest to optimize highest signal-to-noise ratio relative to negative control (mock IgG or negative-location control). For example, Anti- trimethyl histone H3 (Lys 4) (Cat. No. 17-614) can perform successful ChIP of as few as 1 X 10^4 HeLa cells. This protocol is written for simplicity using 1 X 10^5 cells per ChIP to ensure optimal performance of the control antibodies.*
    - *Chromatin from other types of culture vessels can be isolated with slight modifications to the protocol.*
2. Prepare 22 mL of 1X PBS (2.2 mL 10X PBS and 19.8 mL water) for each 150 mm culture dish. Store on ice for washes in later steps of the protocol. It is important that this 1X PBS is ice cold.
3. Thaw the 200X Protease Inhibitor Cocktail III at room temperature for later use. This product contains DMSO and will remain frozen below 18.4°C.
    - *It is recommended to aliquot and store unused Protease Inhibitor Cocktail III at -20°C.*
4. Add 550 µL of 37% formaldehyde (or 1100 µL of 18.5% formaldehyde) directly to 20 mL of growth media to crosslink. Gently swirl dish to mix.
    - *Final concentration of formaldehyde is 1%. Use high quality (molecular-biology grade) formaldehyde. Formaldehyde is typically stabilized with methanol. Upon evaporation of methanol, a white precipitate is observed.* ***Do not use formaldehyde if white precipitate is visible in the solution.***
    - *If using suspension cells, formaldehyde can be added to the media for a final concentration of 1%. However, depending upon the volume of cell culture, there might not be enough 10X glycine. It is recommended to gently pellet the cells and resuspend the cell pellet in 20 mL PBS or media for every 1 x 10^7 cells before adding formaldehyde.*
5. Incubate at room temperature (18-25ºC) for 10 minutes.

    Agitation of cells is not necessary. Performing cross linking in low serum conditions with culture media or PBS is optional, as an optimization parameter to improve cross linking efficiency. Cross linking time can be increased but may result in higher non-specific association of DNA with the ChIP antibody of interest.
6. During the 10 minute incubation, prepare 1X protease inhibitor in PBS: Add 1 mL of ice cold 1X PBS to a conical tube for every dish and add 5 µL of the 200X Protease Inhibitor Cocktail III. Store on ice.
    - *Please note that Protease Inhibitor Cocktail III contains DMSO and will remain frozen below 18.4°C.*

- *This step is only required for adherent cells that must be scraped from the plate. This step and preparation of this buffer is not required for suspension cells.*

7. Add 2 mL of 10X glycine to each dish to quench excess formaldehyde.
    - *If using suspension cells, make sure to add sufficient 10X glycine for a final concentration of 1X glycine.*
8. Swirl for 5 minutes at room temperature.
9. Place dishes on ice.
10. Aspirate media, removing as much medium as possible, being careful not to disturb the cells.
    - *If using suspension cells, spin down cells at 1350 X g for 5 min. then carefully aspirate media.*
11. Add 10 mL of cold 1X PBS prepared in step 2 to wash cells. .
12. Carefully remove 1X PBS and repeat wash.
    - *If using suspension cells, spin down cells at 1350 X g for 5 min. carefully aspirate media and repeat wash.*
    - *Be sure to rinse the cells twice. After second rinse, cell pellets can be snap-frozen or you can continue on to cell lysis and sonication (section C of this protocol).*
13. Add 1 mL of 1X Protease Inhibitor Cocktail III in PBS prepared in Step 6.
14. Scrape cells from each dish into a separate 1.5 mL microcentrifuge tube.
15. Spin at 800 x g at 4°C for 5 minutes to pellet cells.
16. Carefully remove supernatant to avoid aspiration of cells. Cell pellet can be snap-frozen in liquid nitrogen and stored at -80° C at this point, or continue on to cell lysis and sonication.

II. Fresh tissue

1. Dissect non-fixed fresh tissue. Transfer tissue sample into a 50 mL conical tube and wash twice with 30 mL ice cold 1X PBS. Weigh tissue and record weight (you will use this value to determine volume of buffer to use in section C of this protocol).
    - *A piece of tissue approximately 5 mm^3 contains around 10^7 cells and should be sufficient for 100 ChIP samples. Although the mass will depend on the cellularity of the tissue, 5 mm^3 of tissue is approximately 100-200 mg by mass.*
    - *Carefully handle and promptly process all tissue samples to preserve specimen integrity.*
2. Place sample in a tissue culture plate containing 10 mL ice cold 1X PBS. Use a clean razor blade to cut a piece of tissue (around 5 mm^3) into small pieces (typically 1mm^3 or smaller) to improve crosslink efficiency. Alternatively, a plug of tissue from cryosectioned non-formalin-fixed-paraffin-embedded (FFPE) material can be used to obtain a small sample of interest (please see the Magna ChIP™ G Tissue Kit manual, Cat. No. 17-20000, for additional details).
3. Transfer tissue sample to a 50 mL tube.
4. Spin 50 mL tube at 800 x g at 4°C for 5 minutes to pellet sample then, remove supernatent.
5. Resuspend sample in 20 mL ice cold PBS and add 550 µL of 37% formaldehyde (or 1100 µL of 18.5% formaldehyde) to crosslink. Gently swirl tube to mix.
6. Incubate at room temperature for 15 minutes.
7. Add 2 mL of 10X glycine to quench excess formaldehyde. Mix and incubate at room temperature for 5 minutes.
8. Spin at 800 x g at 4°C for 5 minutes to pellet the sample.
9. Wash twice with 20 mL ice cold 1X PBS, then resuspend in 5mL cold 1X PBS.
10. Homogenize the sample several times using a chilled Dounce homogenizer (loose pestle) on ice.
11. Transfer homogenate to a 15 mL conical tube and spin at 800 x g at 4°C for 5 minutes to pellet cells, and carefully remove supernatant.

12. Cell pellet can be frozen and stored at -80°C for future use or immediatly used for next step

C. Cell Lysis to Release Cross-Linked Proteins/DNA

1. Prepare 0.5 mL of Nuclei Isolation Buffer containing 2.5 µL of 200X Protease Inhibitor Cocktail III for each cell pellet.
    - *For tissue use 100 µL lysis buffer for every 10 mg of tissue.*
2. If necessary, thaw cell pellets that have been stored at -80°C.
3. Resuspend cell pellet in Nuclei Isolation Buffer (prepared in Step 1).
4. Incubate on ice for 15 minutes: vortex the cell suspension at high speed for 10 second every 5 minutes to enhance cell breaking.
5. (Optional) At the end of the incubation, homogenize the cell suspension 10 times in a Dounce homogenizer (loose pestle) to facilitate the release of the nuclei.
6. Spin the cell suspension at 800 x g at 4°C for 5 minutes.
7. During 5 minute spin, prepare 0.5 mL of SCW Buffer containing 2.5 µL of 200X Protease Inhibitor Cocktail III for each microcentrifuge tube
8. Remove supernatant. Resuspend cell pellets in 0.5 mL of SCW Buffer containing 2.5 µL of 200X Protease Inhibitor Cocktail III (from Step 7).
    - *For every 1 x 10^7 HeLa cells, 0.5 mL of SCW Buffer is recommended when using this protocol. It is recommended that cell concentration be less than 2 x 10^7 cells/mL, as the ratio of lysis buffer to cell density is important for reliable cell lysis.*
    - *For tissue, use same volume of SCW Buffer as was used for Nuclei Isolation (step 1 above).*
9. If optimal conditions for sonication have already been determined, proceed to Section D. Otherwise see Appendix A for guidelines on optimization of sonication conditions.

D. Sonication of Isolated Chromatin to Shear DNA

Important: Optimal conditions need to be determined to shear cross linked DNA to ~200-1000 base pairs in length, or ~200-600 base pairs for NGS experiments. See Appendix A for a typical protocol. Once shearing conditions have been optimized, proceed with the steps below.

1. If desired, remove 5 µL of cell lysate from Section C, Step 8, for agarose gel analysis of unsheared DNA.
2. Sonicate cell lysate on wet ice (ice-water mixture).
    - *The efficiency of sonication depends upon cell type, cell equivalents and instrumentation. When possible, consult your instrument manufacturer's guidelines for instrument operation. An example of sonicated HeLa cell chromatin fractionated suitably for use with Magna ChIP HiSens kit is shown in Figure 3.*
    - *Keep cell lysate ice cold. Sonication produces heat, which can denature the chromatin. Allow at least 30 seconds between cycles of sonication to prevent sample overheating.*
3. Spin at 10,000 x g at 4°C for 10 minutes to remove insoluble material.
4. Prepare a 5 µL aliquot for agarose gel analysis of the sheared DNA according to the protocol in Appendix A , Steps VII to X. Store on ice if gel analysis will be done in the same day; otherwise store aliquot at - 20ºC.
    - *It is important to perform this analysis to ensure chromatin is sheared to appropriate size.*
5. Remove supernatant and place 50 µL aliquots into new microcentrifuge tubes.
6. If 1 x 10^7 cells were used for chromatin preparation, each 50 µL aliquot contains ~1 x 10^6 cell equivalents of chromatin. This is sufficient for up to 10 immunoprecipitations (100,000 cell equivalents of chromatin per ChIP).
7. Sheared cross linked chromatin can be stored at -80°C for up to 3 months.

E. Immunoprecipitation (IP) of Cross Linked Protein/DNA

Prior to starting this section:

- Remove 200X Protease Inhibitor Cocktail III and thaw at room temperature. Please note that this solution contains DMSO and will remain frozen below 18.4°C.
- Always add protease inhibitors to all buffers before use unless directed otherwise. All buffers should be chilled on ice before use.
- When preparing magnetic beads for immunoprecipitation, it is recommended X + 1 reactions of magnetic beads per ChIP reaction be prepared. To accommodate this suggestion, 250 µL of Magna ChIP A/G beads are provided. Additional Magna ChIP A/G beads are available separately (Cat# 16-663 and 16-663X).

1. Add appropriate amount of 200X Protease Inhibitor Cocktail III to SCW buffer (800 µL SCW buffer plus 4 µL of 200X Protease Inhibitor Cocktail III is required per ChIP reaction).
2. Gently shake the Magna ChIP protein A/G Magnetic Beads vial to resuspend any magnetic particles that may have settled. Dispense the appropriate volume of Magna ChIP protein A/G Magnetic Beads (10 µL per ChIP reaction) to a microcentrifuge tube then place the tube on a magnetic separator (e.g. Magna GrIP Rack cat# 20-400) for 1 minute.
3. Remove supernatant making sure not to aspirate any magnetic beads. Add five times the original bead volume of SCW buffer as appropriate for the number of ChIP samples (50 µL SCW buffer per 10 µL beads). Remove microcentrifuge from the magnetic separator and mix the beads by gently pipetting up and down several times to completely resuspend beads. Place the tube on the magnetic separator for 1 minute.
4. Repeat Step 3 and remove the supernatant, taking care not to disturb the magnetic beads.
5. Resuspend beads in appropriate volume of SCW buffer (10 µL per ChIP reaction).
6. For each chromatin immunoprecipitation, add ~190 µL cold SCW buffer containing Protease Inhibitor Cocktail III (prepared in step 1 above), 10 µL of resuspended beads and the appropriate quantity of antibody to a microcentrifuge tube. The final volume of the chromatin immunoprecipitation reaction should be 200 µL.
    - *For best results, the amount of antibody used per ChIP should be experimentally determined or multiple immunoprecipitations using different amounts of antibody should be performed. For most antibodies testing a range of 1-10 µg of purified antibody is generally appropriate for a standard immunoprecipitation.*
    - *It is highly recommended you perform a negative control ChIP using normal IgG or no antibody.*
    - *To evaluate consistency of IP reactions it is advised that technical replicates for each antibody/chromatin combination be run in each experiment.*
7. Place microcentrifuge tubes on a rotating platform at 50-100 rpm for two hours at 4°C, to keep beads from settling out of solution.
8. Briefly spin the microcentrifuge tubes then place tubes on the magnetic separator for 1 minute.
9. Remove supernatant, taking care not to disturb the magnetic beads, and then add 500 µL cold SCW buffer containing Protease Inhibitor Cocktail III and 5 µL chromatin (100,000 cell equivalents from section D step 6) to each microcentrifuge tube.
10. Save 5 µL of undiluted chromatin (from D section D step 6) in a microcentrifuge tube and store at -20°C; this will be used as 100% input in step 19 below.
11. Place the microcentrifuge tube on a rotating platform at 50-100 rpm overnight at 4°C.
    - *The incubation time could be reduced to as short as 2 hours for high affinity antibodies directed towards more abundant proteins such as histone modifications and RNA polymerase.*
12. Spin the microcentrifuge tube briefly, and then place on the magnetic separator for 1 minute.

13. Remove supernatant, being careful not to disturb the magnetic beads.

14. Add 500 µL cold SCW buffer containing Protease Inhibitor Cocktail III; remove the microcentrifuge tube from the magnetic separator and mix the beads by gently pipetting several times or by gentle flipping the microcentrifuge tube several times. Place the microcentrifuge tube on a magnetic separator for 1 minute then remove supernatant.
15. Repeat wash with 500 µL cold SCW buffer twice (as described in Step 14). Vacuum aspirator should not be used with the low stringency IP wash as bead loss may occur.
16. Perform a final wash with 500 µL cold Low Stringency IP Wash Buffer containing Protease Inhibitor Cocktail III (as before, Step 14).
17. Resuspend the beads in 500 µL cold Low Stringency Buffer containing Protease Inhibitor Cocktail III then transfer to a new microcentrifuge tube. Place the tube on the magnetic separator for 1 minute then remove the supernatant.
18. For ChIP samples, resuspend beads in 50 µL ChIP Elution Buffer, and add 1 µL Proteinase K.
19. To the 5 µL of input chromatin saved from step 10, add 45 µL ChIP Elution Buffer and 1 µL Proteinase K.
20. Incubate the samples in a Thermomixer at 65°C for 2 hours and then at 95°C for 15 minutes. Let the sample cool to room temperature.
21. Briefly spin the microcentrifuge tube briefly to settle contents; then place the tube on the magnetic separator for 1 minute. Carefully transfer 45 µL of the supernatant to a new microcentrifuge tube; not to transfer beads.

F. Real-Time Quantitative PCR to Verify Chip DNA Enrichment

The success of ChIP can be evaluated through qPCR. Verification of ChIP enrichment can be performed using the relative standard curve method of qPCR analysis to compare DNA from a mock IP vs. DNA immunoprecipitated using your ChIP antibody, or can alternatively be compared using the comparative Ct (ΔΔCt) method with two PCR amplicons, a positive control binding region, and a negative control location region. Input DNA is required whether using relative standard curve method or the comparative Ct (ΔΔCt) method. An example of significant enrichment is shown in Figure 1.

1. Add 2 µL of the sample to a PCR plate suitable for your real time instrument of choice (not supplied).
    - *2.5 µL or less ChIP DNA is recommended for a 25 µL PCR reaction.*
    - *Performing triplicate of qPCR reactions per ChIP sample is also recommended.*
    - *If using the relative standard curve method, perform four 5- or 10-fold serial dilutions using the reverse cross linked DNA from the 100% input sample (section D, step 19), and use these samples to build a standard curve. Concentration of the ChIP samples can be calculated as percent of input using the standard curve. Alternatively, data can be calculated in relation to cell equivalents of chromatin, or mass of purified DNA, if desired.*
2. Prepare a qPCR mastermix as shown in Table I. Prepare a sufficient volume of mastermix for one extra tube to account for pipette carryover.
3. Add 23 µL of qPCR mastermix to the 2 µL of the sample.
4. Use caps or an optical tape to seal the plate and start the qPCR reactions.

Table I. qPCR reagent setup and running parameters

qPCR reagent assembly for 1 reaction:		**qPCR parameters:**
ddH_2O	9.5 µl	Initial Denature 94°C 10 min

SYBR®-Green Master Mix 12.5 μl Primer mix 1 μl Total 23 μl	Denature 94°C 20 sec Anneal and Extension: 60°C 1 min } 50 times

G. Data Analysis

There are many algorithms to analyze ChIP result; the two most common methods are the relative standard curve method and the ΔΔCt method.

I. Normalize DNA concentration to percent of input using relative standard curve

1. For each locus of interest, perform a serial dilution with the 100% input sample, perform quantitative real-time PCR with these input samples, ChIP DNA samples, and control samples (IgG, non- immunized serum, or no antibody control).
2. Calculate the threshold cycle (Ct) values using real-time detection system software from qPCR equipment manufacturer.
3. Use the threshold cycle (Ct) values of these input samples to build a standard curve.
4. Determine the concentration (C) of the ChIP DNA as percent of input using the standard curve.
5. Determine the fold enrichment by calculating the ratio of $C_{\text{Ab of interest}}$ and C_{IgG}.
6. For each independent experiment, we suggest that you perform the following ChIP qPCR assays in triplicates in the same plate, if possible.
   - *For the positive control experiment, the antibody of interest is the Anti-Trimethyl-Histone H3 (Lys4) antibody provided in the kit, the locus of interest is the GAPDH promoter region (primers provided) and the negative control locus is the promoter region of a transcriptionally inactive gene such as β globin (not provided).*

ChIP DNA	Negative Control Locus	Locus of Interest 1	Locus of Interest 2	…	Locus of Interest N
Input dilution series 1	X	X	X		X
Input dilution series 2	X	X	X		X
Input dilution series 3	X	X	X		X
Input dilution series 4	X	X	X		X
ChIP with antibody of interest	X	X	X		X
ChIP with negative control antibody (IgG/NIS)	X	X	X		X

II. ΔΔCt method

1. Perform quantitative real-time PCR with 2µL of ChIP DNA, and input DNA in triplicates.
2. Perform quantitative real-time PCR with primer set targeting a positive locus and primer set targeting a negative locus separately.
3. Calculate the threshold cycle (Ct) values using real-time detection system software from qPCR equipment manufacturer.
4. Normalize ChIP DNA Ct values to input (ΔCt) for each primer set by subtracting the Ct value obtained for the input DNA from the Ct value for ChIP DNA: $\Delta Ct = Ct_{ChIP} - (Ct_{input}\text{-Log2 [Input Dilution Factor]})$ (Input dilution factor is 1 if using 100% input sample).
5. Calculate the percent of input for each ChIP: $\%Input = 2^{(-\Delta Ct\ [normalized\ ChIP])}$.
6. Normalize positive locus ΔCt values to negative locus (ΔΔCt) by subtracting the ΔCt value obtained for the negative locus from the ΔCt value for positive locus ($\Delta\Delta Ct = \Delta Ct_{positive} - \Delta Ct_{negative}$).
7. Estimate the fold enrichment of the positive locus sequence in ChIP DNA over the negative locus: Fold enrichment $=2^{\Delta\Delta Ct}$.
    - *This estimate is accurate only if the primer efficiency of both primer sets is identical (and preferably 100% efficient), so careful design and validation of the primer sets are essential.*
    - *For each independent experiment, we suggest that you perform the following ChIP qPCR assays in triplicates in the same plate if possible.*
    - *For positive control experiment, antibody of interest is the Anti-Trimethyl-Histone H3 (Lys4) antibody provided in the kit, the locus of interest is the GAPDH promoter region (primers provided) and the negative control locus is the promoter region of an inactive gene such as β globin (not provided).*

ChIP DNA	Negative Control Locus	Locus of Interest 1	Locus of Interest 2	…	Locus of Interest N
Input	X	X	X		X
ChIP with antibody of interest	X	X	X		X
ChIP with negative control antibody (IgG/NIS)	X	X	X		X

H. Next Generation Sequencing & Analysis

Important Note: *It is recommended for ChIP-seq that more cell equivalents per ChIP are utilized to ensure robust recovery of DNA. The amount of chromatin required will depend both on the protein abundance as well as the affinity of your antibody. It is not recommended to use less than 1e6 cell equivalents/ChIP, and samples may need to be pooled to achieve a minimum of 1-10 ng of DNA as recommended in most library construction protocols.*

1. Follow Magna ChIP HiSens protocol up to Section E Step 18 with the exception of using 20 uL or greater of CS207374 Magna ChIP Protein A/G magnetic beads per ChIP.
2. For ChIP samples, resuspend beads in 50 µL ChIP Elution Buffer and add 1 uL of 10 mg/ml RNase A.

    Note: *To the 5 µL of input chromatin saved from step 10, add 45 µL ChIP Elution Buffer and 1 µL of RNAse A.*
3. Incubate the samples in a Thermomixer at 37°C for 30 minutes and then add 1 µL Proteinase K.
4. Continue by following Steps 20 and 21 of Section E.

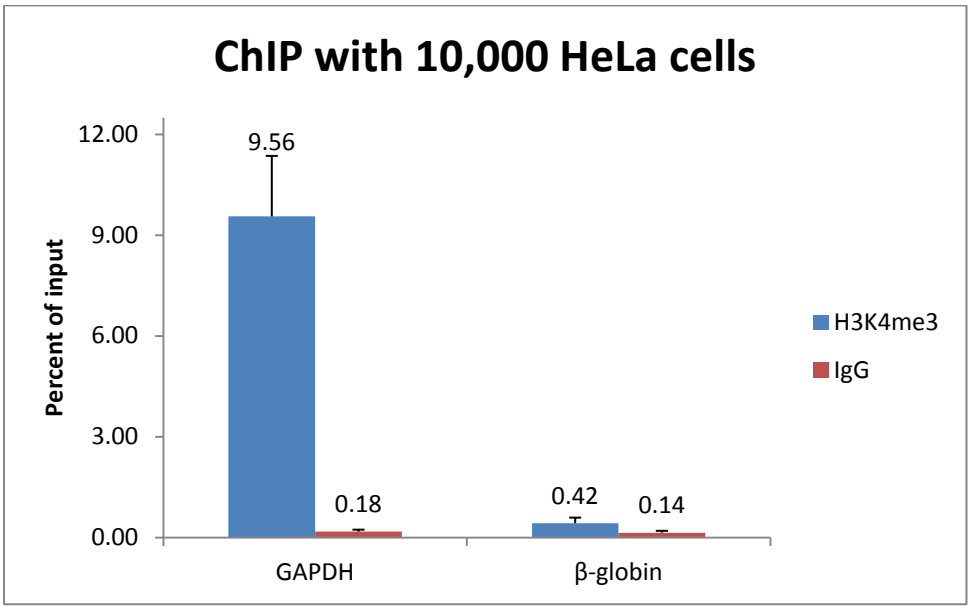
5. Purify DNA by organic extraction, Agencourt Bead cleanup, or PCR clean up columns as desired.

   For organic extraction, transfer to Phase Lock tube that has been microfuged for 1 minute to pellet the locking material. Add an equal volume of phenol. Vortex, microcentrifuge at top speed for 5 min at room temp. Repeat by adding an equal volume of chloroform:isoamyl alcohol, vortexing and microfuging. Transfer aqueous phase to a new epi tube. Add 1/10th volume of 3M Sodium Acetate pH 5.5, 20 ng glycogen and 2,5 volumes of 100% ethanol. Incubate the samples at -80°C for 30 minutes and microcentrifuge at top speed at 4°C for 10 minutes. Aspirate and discard any liquid without disturbing the pellets. Wash the pellets by adding 500 uL of 80% ethanol and spinning at top speed for 5 minutes. Carefully remove and discard any liquid. . Dry the pellets and resuspend in TE.

   For other methods, consult manufacturer's instructions.

6. Continue with NGS library construction step according to manufacturer's protocol.

Performance of Various Antibodies Using Magna ChIP HiSens Kit



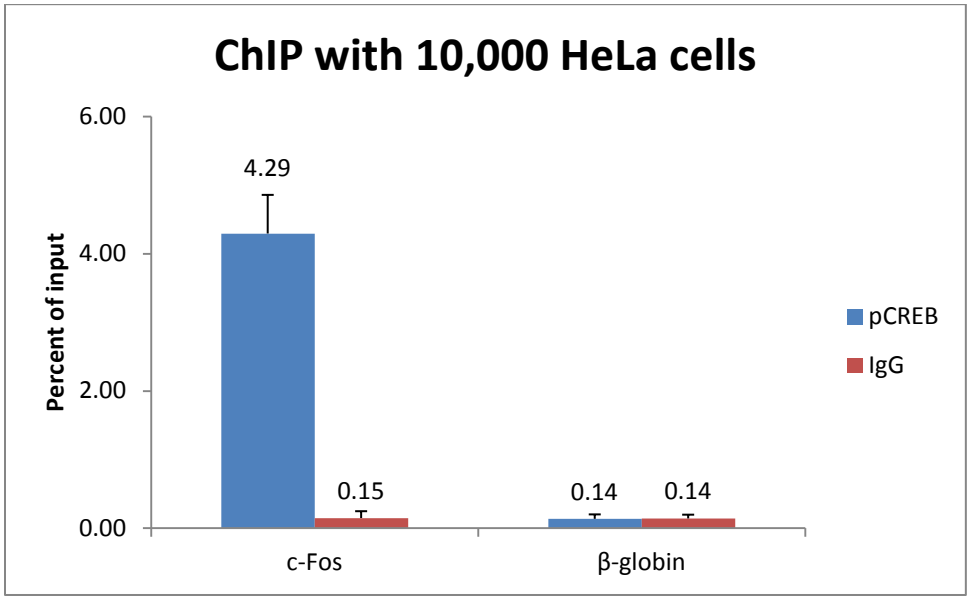


Figure 1: Sonicated chromatin prepared from 10,000 untreated HeLa cells was subjected to chromatin immunoprecipitation using 1 µg of purified IgG (Rabbit IgG: 12-370) or specific antibodies (H3K4Me3: 17-614 (A), Phospho-CREB: 17-10131 (B)) and the Magna ChIP HiSens Kit. Immunoprecipitation of antibody-associated DNA fragments was verified by qPCR using positive control primers (GAPDH promoter primers for H3K4me3 and c-Fos promoter primers for pCREB) and negative control primers (human β-globin promoter primers). Standard deviation of three independent ChIP is shown, and results reflect analysis of 2 µL out of 50 µL total DNA per qPCR reaction.

Performance of Magna ChIP HiSens Kit Using Tissue

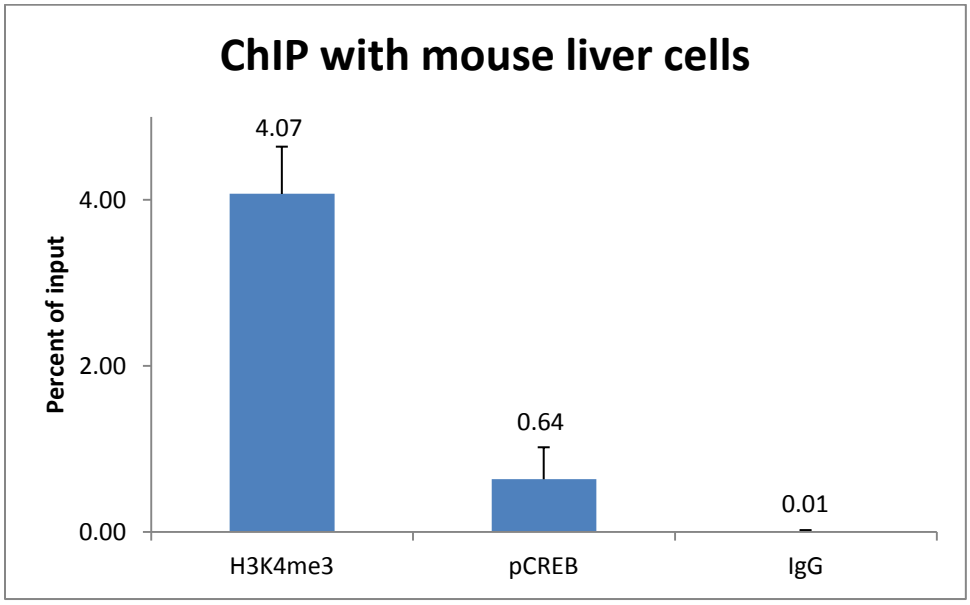


Figure 2: Sonicated chromatin prepared from 0.2 mm^3 mouse liver cells was subjected to chromatin immunoprecipitation using 1 µg of purified IgG (Rabbit IgG: 12-370) or specific antibodies (H3K4Me3: 17-614, Phospho-CREB: 17-10131) and the Magna ChIP HiSens Kit. Immunoprecipitation of antibody-associated DNA fragments was verified by qPCR using positive control primers (mouse GAPDH promoter primers). Standard deviation of three independent ChIP is shown, and results reflect analysis of 2 µL out of 50 µL total DNA per qPCR reaction.

ChIP Optimization and Troubleshooting

Step	Potential Problems	Experimental Suggestions
Cross linking	Not enough or too much cross linking	The amount of formaldehyde and time of cross linking must be determined empirically. Conduct a time course at a fixed formaldehyde concentration and/or investigate a range of formaldehyde concentrations for a fixed time. Note: Histones may not need to be cross linked since they are tightly associated with DNA.
Cell Lysis	Inefficient disruption of cells	It is important to have sufficient Nuclei Isolation Buffer for the number of cells processed. Follow the guidelines in this protocol. Also, verify cell lysis by viewing a 10 µL portion of the cell lysate under the microscope to confirm lack of intact cells.
Chromatin Shearing	Not enough/too much sonication	If fragments are too large or too small optimize sonication conditions using approach outline in appendix A to obtain the appropriate size fragments.
	Denaturation of proteins from overheating sample	Keep the sample on ice during sonication. Shorten the time of each sonication and increase the number of times the sample is sonicated. Allow sufficient time for sample to cool between pulses.
Addition of Primary Antibody	Antibody doesn't recognize protein in fixed chromatin	Choose an antibody directed to a different epitope of the antigen. Decrease the amount of formaldehyde or fixation time in formaldehyde.
	Not enough or too much chromatin	Perform IP from a dilution series of antibody with a fixed amount of chromatin or vice versa.
	Insufficient incubation time	Incubate the antibody of interest with the chromatin at 4°C overnight.
	Low affinity antibody	Select an alternative antibody with higher affinity. Perform a Western blot of the immunoprecipitated protein to verify that the antibody can precipitate the antigen of interest.
	Primary antibody is not compatible with A/G beads	While a wide range of antibodies are compatible with Magna ChIP A/G beads, some antibody isotypes such as IgM or IgY are not. For these types of antibodies, use a bridge antibody or second antibody compatible with A/G beads.
Addition of Secondary Reagent – Protein A	Poor binding of complexes to beads	The magnetic beads settle to the bottom of the tube over time. Be sure Magna ChIP Protein A/G magnetic beads are well mixed prior to removing the appropriate volume for IP.

ChIP Optimization and Troubleshooting *(Continued)*

Step	Potential Problems	Experimental Suggestions
Washing	High background signals due to insufficient washing	Increase number of washes for each wash buffer.
	Low signal due to aspiration of the beads during buffer removal	Carefully remove supernatant and make sure there are no beads in the supernatant prior to removing it. Use rack with magnets capable of firmly holding beads in place (e.g. Magna GrIP Rack Cat. No. 20-400)
Elution and Reversal of cross linking	Incomplete elution	When performing elution, make sure that the temperature is near 60°C. Proteinase K will be inactivated by prolonged incubation at temperatures above 65°C.
	Excessive Cross linking	Excessive cross-linking may not be reversible. Conduct a time course at a fixed formaldehyde concentration and/or investigate a range of formaldehyde concentrations for a fixed time.
PCR	Incorrect Annealing Temperature or Amplification Conditions	Ensure amplification reaction program is correctly set on thermal cycler. Re-examine primers for correct T_m. Perform PCR on genomic DNA to confirm amplification conditions and ability of primers to generate a single DNA product of the expected size.
	More than one PCR product	Redesign primer, perform melting curve.
	Low efficiency in PCR	Redesign using 20-30 nucleotide primers.
	No PCR product	Increase amount of DNA added to the PCR reaction. Increase the number of cycles for the amplification reaction.
	PCR product is a smear	Decrease amount of DNA added to the PCR reaction. Use a hot start *Taq* polymerase to avoid non-specific annealing of primers.

Appendix A: Optimization of DNA Sonication

Optimal conditions for shearing cross-linked DNA to 200-1000 base pairs in length depend on the cell type, cell concentration, and the specific sonicator equipment, including the power settings and duration and number of pulses. Approaches for optimizing sonication may include the following:

A. Varying the concentration of cell equivalents per mL of SCW Buffer with constant sonication parameters.
B. Choosing a fixed concentration of cell equivalents per mL of SCW Buffer and varying cycles and/or power settings of sonication.
C. A combination of both approaches.

The protocol presented below describes optimization following option B and is provided as an example only.

I. Generate a cell lysate by following Section A and B.
II. Continue following the Cell Lysis procedure (section C) through Step 8. Each microcentrifuge tube should contain approximately 10^7 cells in 500 µL of cell lysate.
III. Be sure to keep the samples on wet ice at all times. Sonication generates heat which will denature the chromatin.
IV. Remove 5 µL cell lysate prior to sonication for analysis of unsheared DNA.
V. Shear the chromatin with 4, 8 and 16 pulses at 60% power using a using a Misonix 3000 instrument and a #419 microtip probe, use six 15 second pulses with 50 second intervals between pulses. Keep tubes cool at all times.
VI. Remove 5 µL sonicated chromatin from each condition to a fresh tube.
VII. To all samples (unsheared and sheared), add ChIP Elution Buffer to a final volume of 50 µL.
VIII. Add 1 µL of 10 mg/ml RNAse A and incubate in a thermomixer, water bath or heat block for 30 minutes at 37°C.
IX. Add 1 µL Proteinase K and incubate the samples in a Thermomixer at 65°C for 2 hours and then at 95°C for 15 minutes. Let the sample cool to room temperature.
X. Purify the DNA by PCR clean up column per manufacturers instructions, or using organic extraction as follows:

Transfer to Phase Lock tube that has been microfuged for 1 minute to pellet the locking material.Add an equal volume of phenol. Vortex, then microcentrifuge at maximum speed 5 min at room temp. *Repeat by adding an equal volume of chloroform:isoamyl alcohol, vortexing and microfuging. Transfer aqueous phase to a new epi tube* . Add 1/10th volume of 5M Sodium Acetate, pH 5.5, 20 ng glycogen. *Incubate the samples at -80°C for 30 minutes and microcentrifuge at top speed at 4°C for 10 minutes. Aspirate and discard any liquid without disturbing the pellets. Wash the pellets by adding 500 uL of 80% ethanol and spinning at top speed for 5 minutes. Carefully remove and discard any liquid. . Dry the pellets and resuspend in TE.*

XI. Load 10 µL on a 1-2% agarose gel.
XII. Observe which of the shearing conditions gives a smear of DNA in the range of 200 -1000 bp. See Figure 3 for an example.

XIII. Repeat optimization of the shearing conditions if the results indicate that the resulting DNA is not in the desired size range. Increase the duration or the number of pulses if the chromatin DNA is too large.

DNA Sonication: ChIP DNA Should Be Between 200-1000 bp in Length

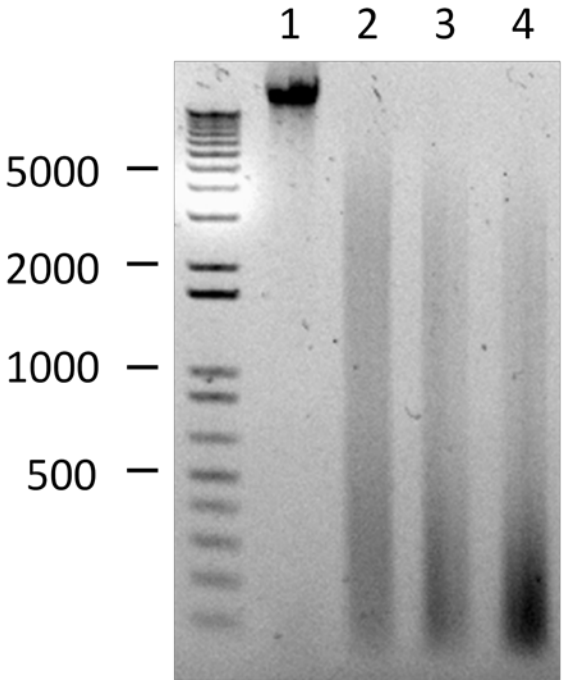


Figure 3: Sheared chromatin from formaldehyde-cross linked HeLa cells was prepared by following Section A, Section B, Section C and Appendix A of the Magna ChIP HiSens Chromatin Immunoprecipitation Kit protocol (cat: 17-10460). 10 µL sheared chromatin was then subjected to electrophoresis through a 2% agarose gel and stained with ethidium bromide. Lane 1: unsonicated chromatin. Lane 2: chromatin sonicated for 4 pulses. Lane 3: chromatin sonicated for 8 pulses. Lane 4: chromatin sonicated for 16 pulses.

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EZ-zyme™ Chromatin Preparation Kit	17-375
Magna ChIP™ G Tissue Kit	17-20000
Magna ChIP™ Protein A+G Magnetic beads (10 reactions)	16-663X
Magna ChIP™ Protein A+G Magnetic beads (50 reactions)	16-663
Magna ChIP™ Protein G Magnetic beads (10 reactions)	16-662X
Magna ChIP™ Protein G Magnetic beads (50 reactions)	16-662
Magna ChIP™ Protein A Magnetic beads (10 reactions)	16-661X
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CpGenome™ 5-mC & 5-hmC Mouse DNA Standards	S8004
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Anti-5-methylcytosine Antibody, clone 33D3	MABE146
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