Histone Targeted ChIP-seq Kit – KOA0880 and KOA0881

Chromatin Immunoprecipitation (ChIP) coupled with high-throughput parallel sequencing as a detection method (ChIP-seq) is one of the primary methods for epigenomic research to investigate protein-DNA interaction on a genome-wide scale. This technique can be used in a variety of life science disciplines including cellular differentiation, tumor suppressor gene silencing, and the effect of histone modifications on gene expression. The histone targeted ChIP-Seq Kit provides optimized reagents, simplified protocols and validation controls which facilitates successful ChIP-seq.

I. KIT COMPONENTS

ChIP-Seq Kit for Histones				
Component	**Catalog Number**	**Size**		**Storage**
		(X10) KOA0880	**(X24) KOA0881**	
Glycine Solution	KOA0880A	175µl	420µl	4°C
Lysis Buffer One	KOA0880B	15mL	36mL	4°C
Lysis Buffer Two	KOA0880C	15mL	36mL	4°C
Shearing Buffer One	KOA0880D	2mL	4mL	4°C
Protease Inhibitor Cocktail One	KOA0880E	30µl	65µl	-20°C
5x ChIP Buffer One	KOA0880F	1.7mL	4mL	4°C
5% BSA (DNA free)	KOA0880G	70µl	175µl	-20°C
Protein-A Coated Magnetic Beads	KOA0880H	240µl	580µl	4°C Do NOT Freeze
Wash Buffer One	KOA0880I	4mL	10mL	4°C
Wash Buffer Two	KOA0880J	4mL	10mL	4°C
Wash Buffer Three	KOA0880K	4mL	10mL	4°C
Wash Buffer Four	KOA0880L	4mL	10mL	4°C
Elution Buffer One (Room temperature)	KOA0880M	6mL	15mL	4°C
Elution Buffer Two	KOA0880N	240µl	580µl	4°C
Rabbit IgG (control isotype antibody)	KOA0880O	10µl	15µl	4°C
H3K4me3-ChIP-seq Grade Antibody	KOA0880P	10µg	10µg	-20°C
GAPDH TSS Primer Pair (positive control)-Human-ChIP-Seq Grade	KOA0880Q	25µl	50µl	-20°C
Myoglobin Exon 2 Primer Pair (negative control)-Human-ChIP-Seq Grade	KOA0880R	25µl	50µl	-20°C
Water-ChIP-Seq Grade	KOA0880S	4mL	10mL	Ambient

DNA Purification Kit			
Component	**Catalog Number**	**Size**	**Storage**
Wash Buffer One w/o Isopropanol	KOA0882G	2mL	4°C
Wash Buffer Two w/o Isopropanol	KOA0882H	2mL	4°C
Buffer C Solution	KOA0882I	4mL	4°C
Magnetic Beads	KOA0882J	400µl	4°C
Carrier Solution	KOA0882D	55µl	-20°C

II. STORAGE AND STABILITY CONDITIONS

Store the components at the indicated temperature upon receipt. Store Protein-A Coated Magnetic Beads and Magnetic Beads at 4°C. DO NOT FREEZE magnetic beads because they may become damaged. Keep the beads in liquid suspension during storage as drying will result in reduced performance.

III. INTRODUCTION

Association between proteins and DNA is a major mechanism in many vital cellular functions such as gene transcription and epigenetic silencing. It is crucial to understand these interactions and the mechanisms by which they control and guide gene regulation pathways and cellular proliferation. Chromatin immunoprecipitation (ChIP) is a technique to analyze the association of proteins with specific genomic regions in intact cells. ChIP can be used to study changes in epigenetic signatures, chromatin remodeling and transcription regulator recruitment to specific genomic sites.

In ChIP assay, living cells are first fixed with a reversible cross-linking agent to stabilize protein-DNA interactions. The most widely used reagent to fix cells is formaldehyde which generates covalent bonds between amino or imino groups of proteins and nucleic acids. Formaldehyde treatment crosslinks both DNA-protein as well as protein-protein complexes.

Following cross-linking, chromatin needs to be sheared very efficiently into homogeneous small fragments that can subsequently be used in immunoprecipitation (IP). The sonication device is most widely used for chromatin fragmentation and provides high quality sheared chromatin ready-to-ChIP. Shearing may also be accomplished with shearing kits which enable an easy shearing process for any cell type. After fragmentation, the sheared chromatin is precipitated with a specific antibody (AB) directed against the protein of interest. The chromatin-AB complex is isolated using magnetic beads. Finally, the precipitated DNA fragments are released from the AB, and analyzed. Enrichment of specific sequences in the precipitated (IP'd) DNA indicates that these sequences were associated with the protein of interest *in vivo*. Analysis of specific regions can be performed by quantitative polymerase chain reaction (qPCR). In recent years, ChIP combined with high-throughput Next-Generation sequencing (ChIP-seq) has become the gold standard for whole-genome mapping of protein-DNA interactions.

IV. REQUIRED EQUIPMENT AND REAGENTS

Equipment:

- Magnetic rack (for 1.5mL microfuge tubes) (part number: **TMS-06** or **TMS-32**)
- Sonication device
- Polymethylpentene microfuge tubes (1.5mL)
- Refrigerated centrifuge for 1.5mL, 15mL and 50mL tubes Cell counter
- Rotator (Rotating wheel)

- Vortex
- Thermomixer
- Qubit system (Invitrogen)
- qPCR cycler

For Tissue:

- Dounce homogenizer with loose and tight fitting pestles
- Scalpels
- Petri dishes (for tissues)

Reagents:

- Gloves to wear at all steps
- Formaldehyde, 37%, Molecular Grade
- Phosphate buffered saline (PBS) buffer (part number: **MB-008**)
- 1 M Sodium butyrate (NaBu) (optional)
- RNase/DNase-free 1.5mL tubes
- 100% isopropanol
- Trypsin-EDTA
- qPCR SYBR® Green Mastermix
- Reagents for library preparation and sequencing
- Quant-IT dsDNA HS assay kit (Invitrogen)

V. KIT METHOD OVERVIEW AND TIME TABLE

ChIP Protocol Overview			
Step	**Description**	**Time needed**	**Day**
1a	Cell collection and DNA-protein cross-linking (for cultured cells)	1 to 2 hours	1
1b	Tissue Disaggregation and DNA-protein cross-linking (for fresh or frozen tissues)		
2a	Cell lysis and chromatin shearing (for cultured cells)	1 to 2 hours	1
2b	Cell lysis and chromatin shearing (derived from tissue sample)		
3	Magnetic immunoprecipitation	Overnight	1-2
4	Elution, de-cross-linking and DNA purification	6 hours	2
5	Quantitative PCR and data analysis prior to Library preparation and Next-Generation Sequencing	2 to 3 hours	3

VI. GENERAL CONSIDERATIONS

1. Cell number

This protocol has been optimized for ChIP on 1,000,000 cells in 300µl ChIP reaction. It is possible to use more cells, however, for optimal performance, we recommend performing separate ChIP assays and pooling the IP'd DNA before purification.

2. Tissue amount (for fresh and frozen tissues)

This protocol has been optimized for ChIP from fresh or frozen mammalian tissues. The chromatin is prepared from 30-40mg of tissue allowing up to 18 ChIP samples (about 1.5 - 2mg of tissue per IP). However, the exact amount of tissue needed for ChIP-seq may vary depending on protein abundance, antibody affinity etc. and should be determined for each tissue type. We recommend performing a pilot experiment.

3. Shearing optimization and sheared chromatin analysis

Before starting the ChIP, the chromatin should be sheared into fragments of 100 to 600 bp. Our kits and protocols are optimized for chromatin shearing using the ChIP sonicator. The maximum volume for shearing is 300µl per 1.5mL Microtube (depending on the specific type). The shearing conditions mentioned in the protocol are adequate for a variety of cell types. However, given that cell types are different, we recommend optimizing sonication conditions for each cell type before processing large quantities of cells or samples. It is important to perform an initial sonication time course experiment to evaluate the extent of chromatin fragmentation.

4. Magnetic beads

This kit includes Protein A-coated magnetic beads. Make sure the beads do not dry during the procedure as this will result in reduced performance. Keep the beads homogenously in suspension at all times when pipetting. Variation in the amount of beads will lead to lower reproducibility. Do not freeze the beads.

The amount of beads needed per IP depends on the amount of antibody used for the IP. The protocol below uses 20µl of beads. The binding capacity of this amount is approximately 5µg of antibody. With most antibodies the recommended amount to use is 1 to 2µg per IP reaction. However, if you plan to use more than 5µg of antibody per IP we recommend increasing the amount of beads accordingly.

5. Negative and positive IP controls

The kit contains a negative (IgG) and a positive (H3K4me3) control antibody. We recommend including one IgG negative IP control in each series of ChIP reactions. We also recommend using the positive control ChIP-seq grade H3K4me3 antibody at least once. The kit also contains qPCR primer pairs for amplification of a positive and negative control target for H3K4me3 (GAPDH-TSS and Myoglobin exon 2, respectively).

6. Quantification

Determine the concentration of the IP'd DNA after the ChIP with a highly sensitive method such as the 'Quant-IT dsDNA HS assay kit' on the Qubit system from Invitrogen. PicoGreen is also suitable but UV spectrophotometric methods such as the NanoDrop are usually not sufficiently sensitive. In most cases it is sufficient to use approximately 10% of the IP'd material for quantification. The expected DNA yield will be dependent on different factors such as the cell type, the quality of the antibody used and the antibody target. The expected DNA yield obtained with the positive control H3K4me3 antibody on 1,000,000 HeLa cells is approximately 10ng.

7. Quantitative PCR

Before sequencing the samples, we recommend analyzing the IP'd DNA by qPCR using at least 1 positive and 1 negative control target. The kit contains a positive and negative control primer pair which can be used for the H3K4me3 positive control antibody in SYBR® Green qPCR assay. Use your own method of choice for analyzing the appropriate control targets for your antibodies of interest.

In order to have sufficient DNA left for sequencing, we recommend using no more than 10% of the total IP'd DNA for qPCR. You can dilute the DNA (1/10 or more) to perform sufficient PCR reactions. PCR reactions should be performed at least in duplicate although performing them in triplicate is recommended to be able to identify potential outliers.

8. Quantitative PCR data interpretation

The efficiency of chromatin immunoprecipitation of particular genomic loci can be expressed as the recovery of that locus calculated as the percentage of the input (the relative amount of immunoprecipitated DNA compared to input DNA). If the amount used for the input was 1% of the amount used for ChIP, the recovery can be calculated as follows:

% recovery = $2^{(Ct_{input} - Ct_{sample})}$

Ct_{sample} and Ct_{input} are the threshold cycles from the exponential phase of the qPCR for the IP'd DNA sample and input, respectively. This equation assumes that the PCR is 100% efficient (amplification efficiency = 2). For accurate results the real amplification efficiency, if known, should be used.

For the positive control antibody (e.g. H3K4me3) the recovery of the positive control target (GAPDH TSS locus) is expected to be between 10 and 20% although this will depend on the cell type used. The recovery of the negative control target (Myoglobin exon 2 locus) should be below 1%.

Criteria to decide whether the sample is good enough for sequencing will be largely target dependent.

Therefore, the following are only general guidelines:

- The recovery of the positive control target should be at least 5%
- The recovery of the negative control target should be below 1%
- The ratio of the positive versus the negative control target should be at least 5

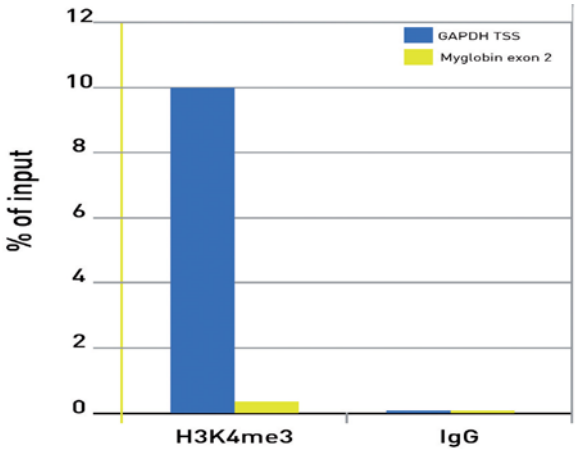

Figure 1. ChIP was performed on human HeLa cells using the control antibodies from the ChIP-seq kit. Sheared chromatin from 1 million cells, 1µl of the positive control antibody and 2µl of the negative IgG control were used per IP. Quantitative PCR was performed with the positive control GAPDH-TSS and the negative control Myoglobin exon 2 primer sets from the kit. The recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

VII. SHORT PROTOCOL FOR EXPERIENCED USERS

Step 1a. Cell collection and DNA-protein cross-linking (for cultured cells)

1. Collect the cells by trypsinization and wash two times with PBS.
2. Count the cells and resuspend them in PBS to obtain up to 10 million cells in 500µl of PBS. Aliquot 500µl of cell suspension in 1.5mL tubes.
3. Add 13.5µl of 37% formaldehyde. Mix by gentle vortexing and incubate for 8 min at room temperature to allow fixation to take place.
4. Stop the fixation by adding 57µl of **Glycine Solution – KOA0880A**. Mix by gentle vortexing and incubate for 5 min at room temperature. Work on ice from this point onwards.
5. Centrifuge at 1,600 rpm (500 x g) for 5 min at 4°C and gently aspirate the supernatant without disturbing the cell pellet.
6. Wash the cells twice with 1mL PBS.

→ Proceed to Step 2a: Cell lysis and chromatin shearing (for cultured cells).

Step 1b. Tissue disaggregation and DNA-protein cross-linking (for fresh or frozen tissue)

7. Weigh 30-40mg of fresh or frozen tissue in a petri dish. Keep samples on ice at all times and minimize the time of manipulation to prevent sample degradation.
8. Chop tissue into small pieces (between 1-3 mm^3) using a scalpel.
9. Add 1mL of ice-cold PBS with protease inhibitors cocktail and disaggregate the tissue using a dounce homogenizer (loose pestle) to get a homogeneous suspension.
10. Transfer the tissue suspension into a 1.5mL tube and centrifuge at 1,300 rpm for 5 min at 4°C. Gently discard the supernatant and keep the pellet.
11. Resuspend the pellet in 1mL of PBS containing 1% of formaldehyde at room temperature.
12. Rotate tube for 8-10 min at room temperature. The fixation time might require an additional optimization. In general, histone marks require shorter fixation (8 min) than transcriptional factors (10-15 min). Please note that stronger fixation may lead to chromatin resistant to sonication.
13. Stop the cross-linking reaction by adding 100µl of glycine. Continue to rotate at room temperature for 5 min.
14. Centrifuge samples at low speed (1,300 rpm) at 4°C.
15. Wash the pellet with ice-cold PBS. Aspirate the supernatant and resuspend the pellet in 1mL of ice-cold PBS plus protease inhibitors.
16. Centrifuge at low speed (1,300 rpm) at 4°C and discard the supernatant.
17. Repeat the washing one more time.

→ Proceed to Step 2b: Cell lysis and chromatin shearing (derived from tissue samples).

Step 2a. Cell lysis and chromatin shearing (for cultured cells)

18. Add 10mL of ice-cold **Lysis Buffer One – KOA0880B** to the cell pellet corresponding to 10 million cells. Resuspend the cells by pipetting up and down several times and incubate for 10 min at 4°C with gentle mixing. Scale down accordingly when using fewer cells.
19. Centrifuge for 5 min at 1,600 rpm (500 x g) and 4°C and discard the supernatant.

20. Add 10mL of ice-cold **Lysis Buffer Two – KOA0880C** to the cell pellet. Resuspend the cells by pipetting up and down several times and incubate for 10 min at 4°C with gentle mixing.
21. Centrifuge for 5 min at 1,600 rpm (500 x g) and 4°C and discard the supernatant.
22. Add **200x Protease Inhibitor Cocktail One– KOA0880E** to the **Shearing Buffer One – KOA0880D**. Keep on ice.
23. Add 1mL of complete Shearing Buffer containing Protease Inhibitor to 10 million cells. Resuspend by pipetting up and down and incubate on ice for 10 min.
24. Shear chromatin by sonication using a sonicator. An initial time course experiment is recommended.
    - Sonicate samples using either high power setting for 10-30 cycles (30 seconds ON, 30 seconds OFF; Stop the system after each run of 10 cycles, vortex and spin down sample) or sonicate for 5-15 cycles (30 seconds ON, 30 seconds OFF) depending on the sonicator. Vortexing is not required between runs.
25. Centrifuge at 13,000 rpm (16,000 x g) for 10 min and collect the supernatant which contains the sheared chromatin.

→ Proceed to Step 3: Magnetic immunoprecipitation

Step 2b. Cell lysis and chromatin shearing (derived from tissue samples)

26. Add 10mL of ice-cold **Lysis Buffer One – KOA0880B** to the pellet corresponding to 30-40mg of tissue. Resuspend the pellet by pipetting up and down and incubate for 10 min at 4°C with gentle mixing.
27. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
28. Add 10mL of ice-cold **Lysis Buffer Two – KOA0880C** to the pellet. Resuspend the pellet by pipetting up and down and incubate for 10 min at 4°C with gentle mixing.
29. Centrifuge for 5 min at 1300 rpm at 4°C and discard the supernatant.
30. Resuspend the pellet in 1.8mL of **Shearing Buffer One – KOA0880D** containing protease inhibitors cocktail and homogenize using a dounce homogenizer (tight pestle).
31. Split the samples into 300µl aliquots in 1.5mL sonication tubes and incubate on ice for 10 min.
32. Shear chromatin by sonication. An initial time course experiment is highly recommended.
    - Sonicate samples using either high power setting for 10-30 cycles (30 seconds ON, 30 seconds OFF; Stop the system after each run of 10 cycles, vortex and spin down sample) or sonicate for 5-15 cycles (30 seconds ON, 30 seconds OFF) depending on the sonicator. Vortexing is not required between runs.
33. Transfer samples to new 1.5mL tubes and centrifuge at 13,000 rpm for 10 min.
34. Collect the supernatant which contains the sheared chromatin.
35. Take an aliquot of 100µl for assessment of chromatin shearing. The remaining chromatin can be stored at -80°C for up to 2 months for further use in the immunoprecipitation.

→ Proceed to Step 3: Magnetic immunoprecipitation

Step 3. Magnetic immunoprecipitation

36. Dilute the **5x ChIP Buffer One – KOA0880F** with **ChIP-seq grade water – KOA0880S** to obtain 1x ChIP Buffer One. Place on ice.
37. Take the required amount of **Protein A-coated magnetic beads - KOA0880H** (20µl/IP) and wash four times with twice the volume of ice-cold 1x ChIP Buffer One.

38. Resuspend the beads after the last wash in the original volume 1x ChIP Buffer One.
39. Set aside 1µl (1%) of the sheared chromatin to use as input sample and keep at 4°C.
40. Prepare the following ChIP reaction mix (1 IP):
    - 6µl of 5% BSA
    - 1.5µl of **200x protease inhibitor cocktail - KOA0880E**
    - 56µl of **5x ChIP Buffer One – KOA0880F**
    - 100µl of sheared chromatin
    - 20µl of **Protein A-coated magnetic beads – KOA0880H**
    - Xµl ChIP-seq grade antibody
    - add **ChIP-seq grade water – KOA0880S** to a total volume of 300µl

If required, NaBu (HDAC inhibitor, 20mM final concentration) or other inhibitors can also be added.

41. Incubate overnight at 4°C on a rotating wheel.
42. The next day, briefly spin the tubes, place them in the ice-cold magnetic rack and discard the supernatant.
43. Add 350µl ice-cold **Wash Buffer One – KOA0880I** and incubate for 5 min at 4°C on a rotating wheel. Discard the wash buffer using the magnetic racks TMS-06 or TMS-32.
44. Repeat the wash as described above once with **Wash Buffer Two – KOA0880J**, **Wash Buffer Three – KOA0880K**, and **Wash Buffer Four – KOA0880L**, respectively.

Step 4. Elution, de-cross-linking and DNA purification

45. After removing the last wash buffer, add 400µl of **Elution Buffer One – KOA0880M** to the beads and incubate for 30 min on a rotating wheel at room temperature.
46. Briefly spin the tubes and place them in a magnetic rack. Transfer the supernatant to a new tube and add 16µl of **Elution Buffer Two – KOA0880N**. Also add 399µl **Elution Buffer One – KOA0880M** and 16µl of **Elution Buffer Two – KOA0880N** to the 1µl input sample. Incubate for 4 hours or overnight in a thermomixer at 1300 rpm at 65°C.
47. Purify the DNA using the DNA purification kit.
48. Add 2µl of **Carrier Solution – KOA0882D** to each IP and input sample. Vortex briefly.
49. Add 400µl of 100% isopropanol to each IP and input sample.
    ATTENTION: Following the addition of isopropanol, the solution may become cloudy. This is not detrimental to your experiment and will not influence the quality or quantity of your purified DNA.
50. Resuspend the provided magnetic beads and transfer 15µl to each IP and input sample.
51. Incubate IP and input samples for 1 hour at room temperature on a rotating wheel (40 rpm).
52. Prepare the Wash Buffer with Isopropanol – One containing 50% isopropanol as follows:

Wash Buffer with Isopropanol - One		
Components	**24 reactions**	**100 reactions**
Wash Buffer One w/o Isopropanol – KOA0882G	2mL	8mL
Isopropanol (100%)	2mL	8mL
Total Volume	4mL	16mL

53. Briefly spin the tubes, place the tubes in a magnetic rack, wait 1 min and discard the buffer. Add 100µl Wash Buffer with Isopropanol – One in each tube. Close the tubes, incubate for 30 seconds at room temperature on a rotating wheel (40 rpm).
54. Prepare the Wash Buffer with Isopropanol – Two containing 50% isopropanol as follows:

Wash Buffer with Isopropanol - Two		
Components	**24 reactions**	**100 reactions**
Wash Buffer Two w/o Isopropanol – KOA0882H	2mL	8mL
Isopropanol (100%)	2mL	8mL
Total Volume	4mL	16mL

55. Briefly spin the tubes, place tubes in a magnetic rack, wait 1 min and discard the buffer. Keep the captured beads and add 100µl Wash Buffer with Isopropanol - Two per tube. Close the tubes, resuspend the beads and incubate for 5 min at room temperature on a rotating wheel (40 rpm).
56. Briefly spin the tubes and place them in a magnetic rack, wait 1 minute and discard the buffer. Keep the captured beads and add 25µl **Buffer C Solution – KOA0882I** per tube (alternatively, a higher volume can be used for the elution if necessary). Close the tubes, resuspend the beads and incubate for 15 minutes at room temperature on a rotating wheel (40 rpm). Resuspend the pelleted beads using the pipet and make sure that you drop them on the bottom of the tube.
57. Spin the tubes and place them in a magnetic rack, wait 1 min and transfer the supernatants into a new labelled 1.5mL tube. Keep the bead pellets on ice.
58. Repeat the elution of the bead pellets for 15 minutes at room temperature on a rotating wheel (40 rpm) in 25µl buffer solution.
59. Spin the tubes and place them in a magnetic rack, wait 1 min and pool the supernatant with the corresponding IP or input sample (1.5mL tube). Discard the beads.
60. Place the DNA on ice and proceed to any desired downstream application, or store the DNA at -20°C or -80°C until further use.

Step 5. Quantitative PCR analysis

Prepare the qPCR mix as follows (20µl reaction volume using the provided control primer pairs):

- 10µl of a 2x SYBR® Green qPCR master mix
- 1µl of primer mix
- 4µl of water
- 5µl IP'd or input DNA

Use the following PCR program:

- 3 to 10 min denaturation step at 95°C (please check carefully supplier's recommendations about Taq polymerase activation time), followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 60°C and 30 seconds at 72 °C. These conditions may require optimization depending on the type of Master Mix or qPCR system used.

VIII. DETAILED PROTOCOL

Step 1a. Cell collection and DNA-protein cross-linking (for cultured cells)

The protocol below is intended for adherent cells. Collect suspension cells by centrifugation and continue with the protocol starting from step 6.

1. Pre-warm PBS, culture medium and trypsin-EDTA at 37°C.
2. Remove the medium and rinse the cells with pre-warmed PBS (10mL for a 75 cm2 culture flask). Gently shake the flask for 2 min.
3. Remove the PBS and add sterile trypsin-EDTA to the culture flask to detach adherent cells from the bottom. Table 4 shows the required amount of trypsin for different numbers of cells. Gently shake the culture flask for 1-2 min or until the cells start to detach. The time needed may depend on the cell type. Do not continue trypsin treatment longer than necessary as prolonged treatment with trypsin may damage the cells. Regularly check if the cells start to detach.

Trypsin-EDTA Volume for Adherent Cells	
Number of cells	**Trypsin-EDTA**
3x 10^6	1mL
1x10^7	3mL
5x 10^7	15mL

4. Immediately add fresh culture medium to the cells when they are detached (see table below). This will inactivate trypsin. Transfer cell suspension to a 50mL tube.

Culture Medium Volume for Adherent Cells	
Number of cells	**Culture medium**
3x 10^6	2mL
1x10^7	6mL
5x 10^7	30mL

5. Rinse the flask by adding 10mL of PBS. Add this volume to your 50mL tubes containing cells from point 4 above.
6. Centrifuge for 5 min at 1600 rpm and 4°C and remove the supernatant.
7. Resuspend the cells in 20mL of PBS and count them. Collect the cells by centrifugation for 5 min at 1600 rpm and 4°C and remove the supernatant.
8. Resuspend the cells in PBS to obtain a concentration of up to 10 million cells per 500µl of PBS. If desired, the cell concentration can be decreased down to 1 million per 500µl. Label 1.5mL tubes and aliquot 500µl of cell suspension in each tube.
9. Add 13.5µl of formaldehyde 37% to each tube containing 500µl of cell suspension. Mix by gentle vortexing and incubate for 8 min at room temperature to allow fixation to take place.
10. Add 57µl of **Glycine Solution – KOA0880A** to the cells to stop the fixation. Mix by gentle vortexing and incubate for 5 mins at room temperature. Keep cells on ice from this point onwards.
11. Collect the cells by centrifugation at 1600 rpm for 5 min and 4°C. Discard the supernatant without disturbing the cell pellet.
12. Wash the cells twice with 1mL of cold PBS.

→ Proceed to Step 2a: Cell lysis and chromatin shearing (for cultured cells).

Step 1b. Tissue disaggregation and DNA-protein cross-linking (for fresh or frozen tissue)

→ Proceed to Step 3: Magnetic immunoprecipitation

13. Weigh 30-40mg of fresh or frozen tissue in a petri dish. Keep samples on ice at all times and minimize the time of manipulation to prevent sample degradation.
14. Chop tissue into small pieces (between 1-3 mm^3) using a scalpel blade.
15. Add 1mL of ice-cold PBS with protease inhibitors cocktail and disaggregate the tissue using a dounce homogenizer (loose pestle) to get a homogeneous suspension.
16. Transfer the tissue suspension into a 1.5mL tube and centrifuge at 1300 rpm for 5 min at 4°C. Gently discard the supernatant and keep the pellet.
17. Resuspend the pellet in 1mL of PBS containing 1% of formaldehyde at room temperature.
18. Rotate tube for 8-10 min at room temperature.
19. The fixation time might require an additional optimization. In general, histone marks require shorter fixation (8 min) than transcriptional factors (10-15 min). Please note that stronger fixation may lead to chromatin resistant to sonication.
20. Stop the cross-linking reaction by adding 100µl of **Glycine Solution – KOA0880A**. Continue to rotate at room temperature for 5 min.
21. Centrifuge samples at low speed (1300 rpm) at 4°C.
22. Wash the pellet with ice-cold PBS. Aspirate the supernatant and resuspend the pellet in 1mL of ice-cold PBS plus protease inhibitors.
23. Centrifuge at low speed (1300 rpm) at 4°C and discard the supernatant.
24. Repeat the washing one more time.

→ Proceed to Step 2b: Cell lysis and chromatin shearing (derived from tissue samples)

Step 2a. Cell lysis and chromatin shearing (for cultured cells)

25. Add 1mL of ice-cold **Lysis Buffer One – KOA0880B** to the 1.5mL tube containing 10 million cells. Resuspend the cells by pipetting up and down several times and transfer them to a 15mL tube. Add 9mL of **Lysis Buffer One – KOA0880B** and incubate for 10 min at 4°C with gentle mixing. If the starting amount of cells was less than 10 million, scale down accordingly (e.g. use a total of 5mL Buffer One for 5 million cells).
26. Pellet the cells by centrifugation at 1,600 rpm for 5 min and 4°C and discard the supernatant.
27. Add 1mL of ice-cold **Lysis Buffer Two – KOA0880C** and resuspend the cells by pipetting up and down several times. Add another 9mL of **Lysis Buffer Two – KOA0880C** and incubate for 10 min at 4°C with gentle mixing. Scale down accordingly when using less than 10 million cells.
28. Pellet the cells again by centrifugation for 5 min at 1,600 rpm (500 x g) and 4°C and discard supernatant.
29. Add **200x Protease Inhibitor Cocktail – KOA0880E** to **Shearing Buffer One – KOA0880D**. Prepare 1mL of Complete Shearing Buffer per tube of 10 million cells. Keep on ice.
30. Add 1mL of Complete Shearing Buffer to 10 million cells. Resuspend the cells by pipetting up and down several times. The final cell concentration should be 1 million cells per 100µl Complete Shearing Buffer. Split into aliquots of 100 to 300µl and transfer the cell suspension to 1.5mL tubes. Incubate on ice for 10 min. Vortex and spin down the samples.
31. Split the samples into 300µl aliquots in 1.5mL sonication tubes and incubate on ice for 10 min.
32. Centrifuge at 13,000 rpm (16,000 x g) for 10 min and collect the supernatant which contains the sheared chromatin. Use the chromatin immediately in immunoprecipitation or store it at -80°C for up to 2 months. If desired, the chromatin shearing efficiency can be analyzed at this step.

Step 2b. Cell lysis and chromatin shearing (derived from tissue samples)

33. Add 10mL of ice-cold **Lysis Buffer One - KOA0880B** to the pellet corresponding to 30-40mg of tissue. Resuspend pellet by pipetting up and down, incubate for 10min at 4°C with gentle mixing.
34. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
35. Add 10mL of ice-cold **Lysis Buffer Two – KOA0880C** to the pellet. Resuspend the pellet by pipetting up and down and incubate for 10 min at 4°C with gentle mixing.
36. Centrifuge for 5 min at 1,300 rpm at 4°C and discard the supernatant.
37. Resuspend the pellet in 1.8mL of **Shearing Buffer One – KOA0880D** containing **Protease Inhibitors Cocktail – KOA0880E** and homogenize using a dounce homogenizer (tight pestle).
38. Split the samples into 300µl aliquots in 1.5mL sonication tubes and incubate on ice for 10 min.
39. Shear chromatin by sonication. An initial time course experiment is highly recommended.
    - Sonicate samples using either high power setting for 10-30 cycles (30 seconds ON, 30 seconds OFF; Stop the system after each run of 10 cycles, vortex and spin down sample) or sonicate for 5-15 cycles (30 seconds ON, 30 seconds OFF) depending on the sonicator. Vortexing is not required between runs.
40. Transfer samples to 1.5mL tubes and centrifuge at 13,000 rpm for 10 min.
41. Collect the supernatant which contains the sheared chromatin.
42. Take an aliquot of 100µl for assessment of chromatin shearing. The remaining chromatin can be stored at -80°C for up to 2 months for further use in the immunoprecipitation.

Step 3. Magnetic immunoprecipitation

This protocol has been optimized for 1 million cells per ChIP. Although it is possible to use more cells, we recommend performing separate ChIP reactions and pool the samples before purification of the DNA.

43. Determine the total number of IP's in the experiment. We recommend to include one negative control in each experiment (IP with the IgG negative control – KOA0880O). Take the required amount of **Protein A-coated magnetic beads – KOA0880H** (20µl/IP). Dilute the **5x ChIP Buffer One -KOA0880F** with **ChIP-seq grade water – KOA0880S** to obtain 1x ChIP Buffer One. The total amount of 1x ChIP Buffer One needed is 9 times the volume of beads required for the experiment. Place the diluted ChIP Buffer One on ice.
44. Wash the beads 4 times with twice the volume of ice-cold 1x ChIP Buffer One. To wash the beads, add 1x ChIP Buffer One, resuspend the beads by pipetting up and down several times and place the tubes in the magnetic rack. Wait for one min to allow the beads to be captured by the magnet and remove the supernatant. Repeat this 3 times. Alternatively, centrifuge the tubes for 5 min at 1,300 rpm, discard the supernatant and keep the bead pellet.
45. After the last wash, resuspend the beads in the original volume 1x ChIP Buffer One.
46. Prepare the ChIP reaction mix according to table below. If required, NaBu (20mM final concentration) or other inhibitors can also be added. Use 2µl of the **Rabbit IgG control antibody – KOA0880O** for the negative control IP. If a positive control IP is included in the experiment, use 1µl of the **H3K4me3 ChIP-seq grade control antibody – KOA0880P**. When preparing the reaction mix, place 1µl of the sheared chromatin aside to be used as an input the next day.

ChIP Reaction Mix							
Number of IP's	5% BSA (µl)	200x Protease inhibitor cocktail (µl)	5x Buffer One (µl)	Sheared chromatin (1^{10} cells) (µl)	Magnetic beads (µl)	ChIP-seq grade water (µl)	Antibody (µl)
1	6	1.5	56	100	20	116.5 -x	X
2	12	3	112	200	40	233 -x	X
4	24	6	224	400	80	466 -x	X
6	36	9	336	600	120	699 -x	X
8	48	12	448	800	160	932 -x	X

47. Incubate the tubes overnight at 4°C under constant rotation at 40 rpm on a rotating wheel.
48. The next morning, after the overnight incubation, briefly spin the tubes and place them in the magnetic rack. Wait for one min and remove the supernatant. To wash the beads, add 350µl of **Wash Buffer One – KOA0880I**, gently shake the tubes to resuspend the beads and incubate for 5 min on a rotating wheel at 4°C.
49. Repeat the wash as described above once with **Wash Buffer Two – KOA0880J**, **Wash Buffer Three – KOA0880K**, and **Wash Buffer Four – KOA0880L** using the same buffer volume, respectively.

Step 4. Elution, de-cross-linking and DNA purification

50. After removing the last wash buffer, add 400µl of **Elution Buffer One – KOA0880M** to the beads and incubate for 30 min on a rotating wheel at room temperature.
51. Briefly spin the tubes and place them in a magnetic rack. Transfer the supernatant to a new tube and add 16µl of **Elution Buffer Two – KOA0880N**. Also add 399µl **Elution Buffer One – KOA0880M** and 16µl of **Elution Buffer Two – KOA0880N** to the 1µl input sample. Incubate for 4 hours or overnight in a thermomixer at 1300 rpm at 65°C.
52. Pool samples if necessary.
    - NOTE: Up to 2 samples can be easily pooled. If more than 2 samples need to be pooled, process each sample purification individually, pool final eluates at the end of the DNA purification and concentrate.
53. Add 2µl of **Carrier Solution – KOA0882D** to each IP and input sample.
54. Add 400µl of 100% isopropanol to each IP and input sample. Vortex briefly.
    - Following the addition of isopropanol, the solution may become cloudy. This is not detrimental to your experiment and will not influence the quality or quantity of your purified DNA.
55. Resuspend the provided magnetic beads and transfer 15µl to each IP and input sample.
    - Keep magnetic beads in liquid suspension during storage at 4°C and at all handling steps, as drying will result in reduced performance.
    - The final volume is now 817µl per reaction.
56. Incubate IP and input samples for 1 hour at room temperature on a rotating wheel (40 rpm).

    Prepare the Wash Buffer with Isopropanol – One containing 50% isopropanol as shown in table.

Wash Buffer with Isopropanol - One		
Components	**24 reactions**	**100 reactions**
Wash Buffer One w/o Isopropanol – KOA0882G	2mL	8mL
Isopropanol (100%)	2mL	8mL
Total Volume	4mL	16mL

57. Briefly spin the tubes, place in magnetic rack, wait 1 min and discard the buffer. Add 100µl Wash Buffer with Isopropanol – One per tube. Close the tubes and vortex well until the bead pellet is completely broken. Incubate for 5 minutes at room temperature on a rotating wheel (40 rpm).
    - Always briefly spin the tubes to bring down liquid caught in the lid prior to positioning into the Magnetic Rack.

    Prepare the Wash Buffer with Isopropanol – Two containing 50% isopropanol as shown in table.

Wash Buffer with Isopropanol - Two		
Components	**24 reactions**	**100 reactions**
Wash Buffer Two w/o Isopropanol – KOA0882H	2mL	8mL
Isopropanol (100%)	2mL	8mL
Total Volume	4mL	16mL

58. Wash the IP and input samples with the Wash Buffer with Isopropanol - Two as follows. Briefly spin the tubes, place into the magnetic rack, wait 1 min and discard the buffer. Add 100µl Wash Buffer with Isopropanol - Two per tube. Close the tubes and vortex well until the bead pellet is completely broken. Incubate for 5 minutes at room temperature on a rotating wheel (40 rpm).

NOTE: This Elution Buffer C is suitable for direct qPCR analysis, whole genome amplification, chip hybridization and Next-Generation sequencing.

59. Briefly spin the tubes and place them into the magnetic rack, wait 1 min and discard the buffer. Spin the tubes again and place them on the magnetic rack. Discard the remaining Wash Buffer with Isopropanol - Two if necessary. Resuspend the beads pellet with 25µl of **Buffer C Solution – KOA0882I**. Incubate at room temperature for 15 minutes on a rotating wheel (40 rpm).
60. Spin the tubes and place them into the magnetic rack, wait 1 min and transfer the supernatants into a new labelled 1.5mL tube. Keep the bead pellet on ice.
61. Repeat the elution of the bead pellet for 15 mins at RT on a rotating wheel (40 rpm) in 25µl of buffer solution.
62. Spin the tubes and place them in a magnetic rack, wait 1 min and pool the supernatant with the corresponding IP or input sample (1.5mL tube). Discard the beads.
    - Total elution volume for both IP and input samples is 50µl.
63. Place the DNA on ice and proceed to any desired downstream applications, or store it at -20°C or -80°C until further use.

64. Take 5µl (10%) of IP'd DNA and determine the concentration with Quant-IT dsDNA HS assay kit using the Qubit or a similar method.
65. Store the DNA at -20°C until analysis with qPCR or by high throughput sequencing.

Step 5. Quantitative PCR analysis

Before sequencing the samples, we recommend analyzing the IP'd DNA by qPCR using at least 1 positive and 1 negative control target. The kit contains a **positive (GAPDH TSS) – KOA0880Q** and **negative (Myoglobin Exon 2) – KOA0880R** control primer pair which can be used for the positive control antibody provided in the kit in SYBR® Green qPCR assay using the protocol described below. Use your own method of choice for analyzing the appropriate control targets for your antibodies of interest.

Prepare the qPCR mix as follows (20µl reaction volume using the provided control primer pairs):

- 10µl of a 2x SYBR® Green qPCR master mix
- 1µl of primer mix
- 4µl of water
- 5µl IP'd or input DNA

Use the following PCR program:

- 3 to 10 min denaturation step at 95°C (please check carefully supplier's recommendations about Taq polymerase activation time), followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 60°C and 30 seconds at 72 °C. These conditions may require optimization depending on the type of Master Mix or qPCR system used.

IX. TROUBLESHOOTING

Critical steps	Troubles, solutions, and comments	
Cross-linking	Cross-linking is too weak.	Make sure you perform the fixation step for the correct period of time, at the right temperature and with the correct formaldehyde concentration. e.g.: incubate for 8 minutes at room temperature with 1% formaldehyde final concentration (weight/ volume). Also, use high quality, fresh formaldehyde.
	Cross-linking is too strong.	
	Proteins have unique ways of interacting with the DNA. Some proteins are not directly bound to the DNA but interact with other DNA-associated proteins.	Very short or very long cross-linking time can lead to DNA loss and/or elevated background, therefore the optimal cross-linking time should be found empirically as maximal specificity and efficiency of ChIP.
	Both cross-linking time and formaldehyde concentration are critical.	Cross-linking can affect both efficiency of chromatin shearing and efficiency of specific antigen immunoprecipitation. Shorter cross-linking times (5 to 10 minutes) and/or lower formaldehyde concentrations (1%, weight/ volume) may improve shearing efficiency while, for some proteins especially those that do not directly bind DNA, this might reduce the efficiency

		of cross-linking and thus the yield of precipitated chromatin.
	The optimal duration of cross-linking varies between cell type and protein of interest.	It is possible to optimize the fixation step by testing different incubation times: such as 10, 20 and 30 minutes. Do not cross-link for longer than 30 minutes as cross-links of more than 30 minutes cannot be efficiently sheared.
	Efficient fixation of a protein to chromatin *in vivo* is a crucial step for ChIP. The extent of cross-linking is probably the most important parameter.	Two major problems concerning the subsequent immunoprecipitation step should be taken into account: 1) an excess of cross-linking can result in the loss of material or reduced antigen availability in chromatin, or both. 2) the relative sensitivity of the antigen epitopes to formaldehyde. It is essential to perform the cross-linking step with care.
	It is essential to quench the formaldehyde.	Use glycine to stop the fixation: quench formaldehyde with 125 mM glycine for 5 minutes at room temperature (add 57µl of 1.25M glycine per 513.5µl of sample. Alternatively, wash the fixed cells properly and make sure you get rid of ALL the formaldehyde.
Cell lysis	Temperature is critical.	Perform cell lysis at 4°C (cold room) or on ice. Keep the samples ice-cold at all times during the cell lysis and use ice-cold buffers.
	Protein degradation during lysis can occur.	Add the protease inhibitors to the lysis buffer immediately before use.
Cell type	Kit protocol validation.	HeLa, NCCIT 293T, Chondrocytes, P19, ASC (adipose stem cells) and Kerationocytes have been used to validate the Magnetic ChIP protocol.
Cell number necessary/ChIP	The amount of cells required for a ChIP experiment is determined by cell type, protein of interest and antibodies used.	You can use from 1,000,000 to 10,000,000 cells per IP.
Chromatin shearing	Optimal shearing conditions are important for ChIP efficiency.	Shearing conditions for each cell type must be optimized from cell collection, fixation to shearing method (settings of the sonicator apparatus).
	Critical points for shearing optimization.	1) Not to start with a too large amount of cells (1x 10^6 cells or less is ok) 2) Keep samples cold (4°C) 3) High % SDS favors better sonication but inhibits immunoselection (optimal range: 0.1 to 1%). Dilutions must be adapted accordingly prior to immunoselection; the final SDS concentration should not be higher than 0.15 to 0.20% (e.g. If the shearing buffer contains 0.75% SDS, the sheared chromatin is diluted 3.5 to 4.0 fold in the [P.I.-ChIP buffer 1x])
	Shear the samples of chromatin using the sonicator.	Maintain temperature of the samples close to 0°C. The chromatin shearing needs to be optimized for each cell type.

Sheared chromatin analysis	Do not load too much DNA on a gel.	Loading of large quantities of DNA on agarose gel can lead to poor quality pictures, which do not reflect the real DNA fragmentation. The DNA amount to load depends on the size of the well and on the gel size.
	Agarose concentration.	Do not use more than 1-1.5% agarose gel and run slowly (Volt/cm and time depend on the gel size).
	Running buffer concentration.	1x TAE or TBE is preferred to 0.5x TAE, which can lead to smears on agarose gel.
Sheared chromatin amounts	How much sheared chromatin do I need to prepare?	Most of the sheared chromatin is to be used in the ChIP experiment, but remember that some of the sheared chromatin is needed as control as it corresponds to the input sample for the ChIP experiment and it can also be checked on agarose gel.
	Dilute the sheared chromatin in ChIP buffer for Immuno-selection incubation.	The sheared chromatin is diluted in complete Buffer A prior to the immunoselection incubation. (Add 870µl of complete Buffer A to the 130µl of sheared chromatin). Dilute the sheared chromatin at least 7 fold. Adjust the ChIP buffer volume added to the chromatin accordingly.
Antibody binding beads	Bead centrifugation.	Don't spin the beads at high speed. Use gentle centrifugation (500 x g for 2-3 minutes) as described in the manual. g = 11.18 x r x (rpm/1000) ^2; knowing that r is the radius of rotation in mm. It is possible to centrifuge the 1.5mL tubes at 1,000 – 2,000 g, for 20 seconds.
	Bead storage	Store at 4°C. Do not freeze
	Antibody binding capacity	Polyclonal antibody from rabbit, guinea pig, pig, human IgG. Monoclonal antibody from mouse (IgG2), human (IgG1,2 and 4); and rat (IgG2c).
Protease inhibitors	Storage	Some inhibitors are unstable in solution. The provided protease inhibitor mix should be kept frozen at -20°C, and thawed before use.
Other enzyme inhibitors	Specific enzyme inhibitors are not included in the kit, such as phosphatase inhibitors.	Add phosphatase inhibitors or others to Buffers A and B, if necessary, depending on your research field and protein(s) of interest to be ChIP'd. Add NaBu for histone ChIPs.
Negative ChIP control (s)	Use non-immune IgG in the IP incubation mix.	Use the non-immune IgG fraction from the same species the antibodies were produced in.
	Do not add antibody to the IP.	Incubation with beads, which were not coated with antibodies could also be used as a negative ChIP control as well as non-immune IgG.

	Use antibody and specifically blocked antibody in parallel.	Use one antibody in ChIP and the same antibody that is blocked with specific peptide. To specifically blocked one antibody: pre-incubate the antibody with saturating amounts of its epitope specific peptide for about 30 minutes at room temperature before use in the IP incubation mix. Use in ChIP, the blocked antibody as a negative control in parallel with the unblocked antibody.
Antibody in IP	How many negative controls are necessary?	If multiple antibodies - of the same specie - are to be used with the same chromatin preparation, then a single negative ChIP control is sufficient for all of the antibodies used.
	Why is my antibody not working in ChIP?	Antibody-antigen recognition can be significantly affected by the cross-linking step resulting in loss of epitope accessibility and/or recognition.
	Which antibody should I use in ChIP?	Use ChIP-grade antibodies. If not available, it is recommended to use several antibodies directed against different epitopes of the same protein. Verify that the antibodies can work directly in IP on fresh cell extracts. Also, when testing new antibodies, include known ChIP-grade antibodies as positive control for your ChIP assay.
	How do I choose an antibody for ChIP?	Be aware of the possible cross-reactivity of antibodies. Verify the antibody specificity by Western blot analysis. Antigen affinity purification can be used to increase titer and specificity of polyclonal antibodies.
	Are my antibodies going to bind the protein A or protein G?	There is a significant difference in affinity of different types of immunoglobulins to protein A or G. Therefore, in function of the antibody used for your ChIP, it is recommended to choose either protein A or protein G coated beads (see table below).
Immuno-selection incubation	What is the best incubation time for immuno-selection using the ultrasonic water bath?	To incubate the sheared chromatin with antibodies for 15 to 30 minutes works for many antibodies, however, the kinetics for reaching equilibrium of epitope-antibody binding may differ for each antibody and target. Optimization might improve the results (e.g. the incubation time may need to be increased for some antibodies).
	How does the immuno-selection work with using the ultrasonic water bath?	The rate-limiting step in many immunoassays is associated with the slow kinetics of binding of macro-molecular antigen to antibody. It was demonstrated that the use of ultrasonic energy to enhance mass transport across liquid/solid interfaces can dramatically accelerate antigen binding to antibodies.
	What are the water bath specifications?	Capacity: 5.5 liters. Size (L x W x H): 29 x 15 x 15 cm. Frequency: 42 kHz. Max power requirement: 130 W. RF-Power: 130 W

	Can I use the kit w/o an ultrasonic water bath?	Yes, then a long incubation at 4ºC should be used. Depending on the antibody and target to be ChIP'd, the time of incubation ranges from 2 to 16 hours and should be determined empirically for each antibody.
Polymerase chain reaction	Primer design	Primer length: 18 to 24 nucleotides/ Primer Tm: 60°C (+/- 3.0ºC)/ % GC: 50% (+/- 4%)
	Controls: Negative and positive	Negative PCR controls: PCR with DNA from samples IP'd with non-immune antibodies (negative IgG). Alternatively, PCR using DNA from ChIP samples and primers specific for a DNA region to which your antigen of interest is not binding. Positive PCR control: PCR using input DNA.
	No PCR signal	Include a positive PCR control as a control for your PCR mix (your primers, dNTP and Master Mix) using the Input DNA or a DNA sample of the same origin.
	High Ct values	Use more input chromatin.
	CtNegCtl and CtTarget	The ratio between target IP and negative control IP depends on the antibody used.
	Background is high	Verify that you properly perform the following steps: Keep the antibody binding beads and DNA purifying slurry in suspension while adding to tubes. Check by eye that equal pellets of beads and slurry are present in each tube. Washes are critical.
	Using end-point PCR rather than quantitative PCR	If gel electrophoresis is used to estimate intensities of PCR products, then relative occupancy of a factor at a locus is the ratio of the intensity of the target IP band to the negative control IP band.
Freezing	Samples can be frozen at several steps of the protocol	Pellets of formaldehyde fixed cells can be stored at - 80°C for at least a year. Sheared chromatin can be stored at -80°C for months, depending on the protein of interest to be ChIP'd. Purified DNA from ChIP and input samples can be stored at -20°C for months.
	Avoid multiple freeze/thaw cycles.	Snap freeze and thaw on ice (e.g.: fixed cell pellets and sheared chromatin).

X. AFFINITY OF PROTEIN A AND PROTEIN G TO DIFFERENT IgG SUBCLASSES

Species	Immunoglobulin isotype	Protein A	Protein G
Human	IgG1	++	++
	IgG2	++	++
	IgG3	-	++
	IgG4	++	++
	IgA	+	-
	IgD	+	-
	IgE	+	-
	IgM	+	-
Mouse	IgG1	+	++
	IgG2a	++	++
	IgG2b	++	++
	IgG3	+	++
	IgM	+/-	-
Rat	IgG	++	++
	IgG1	+/-	+
	IgG2a	+/-	++
	IgG2b	+/-	+
	IgG2c	+/-	+
	IgM	+/-	-
Rabbit	IgG	++	++
Hamster	IgG	+	++
Guinea pig	IgG	++	+
Bovine	IgG	+	++
Horse	IgG	+	++
Sheep	IgG	+/-	++
Goat	IgG	+/-	++
Pig	IgG	++	++
Chicken	IgG	-	+/-

XI. RELATED PRODUCTS

Catalog #	Product Name	Size
600-401-I57	Anti-Histone H3 [Monomethyl Lys4] (RABBIT) Antibody	50µg
MB-064-1000	ELISA Microwell Blocking Buffer with Stabilizer (Azide and Mercury Free)	1L
TMBE-100	TMB ELISA PEROXIDASE SUBSTRATE	100mL
TMS-06	6 position Microcentrifuge Tube TrueBlot® Magnetic Separator	1x6
TMS-15-50	15/50 Tube TrueBlot® Magnetic Separator	1x15 1x50
TMS-32	32 position Microcentrifuge Tube TrueBlot® Magnetic Separator	1x32