Instruction Manual

LowCell# ChIP kit

The Low Cell Number Chromatin Immunoprecipitation kit

Cat. No. **C01010070** (kch-maglow-A16)
C01010071 (kch-maglow-G16)
C01010072 (kch-maglow-A48)
C01010073 (kch-maglow-G48)

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Introduction

Diagenode provides kits with optimized reagents and simplified protocols for ChIP including the LowCell# ChIP Kit, HighCell# ChIP Kit, iDeal ChIP-seq kit and the True MicroChIP kit. This protocol describes the use of the LowCell# ChIP Kit which features:

- One day protocol
- Rapid DNA purification with a simplified DIB (DNA isolation buffer)
- Ability to work with as little as 200 cells for histone ChIPs.
- Reduction of reagent usage with fewer buffers
- Fewer steps and reduced handling with magnetic procedure using our optimized DiaMag Magnetic Rack
- Validation with our shearing kits and the Bioruptor® sonicator
- A variety of control options with our ChIP-Seq and ChIP grade antibodies and negative IgG from rabbit or mouse.

Kit Materials

Kit Content

This kit is sufficient to perform 16 ChIP assays (2 rows of 8 IPs each) or 48 ChIP assays.

Table 1: LowCell# ChIP kit contents

Note: Store components at recommended temperatures.

Low Cell Number ChIP kit				
Component	**Description**	**Quantity (x16)**	**Quantity (x48)**	**Storage**
Buffer A	Detergent mix, salt and ion chelator mix included.	25 ml	75 ml	4°C
Protein A-coated magnetic beads* or **Protein G-coated magnetic beads****	The beads are supplied for 16 IPs; detergent and 0.02% sodium azide included.	220 µl	660 µl	4°C **Do not freeze**
Rabbit IgG* or Mouse IgG **	1 µg/µl	15 µl	45 µl	4°C
1.25 M Glycine	-	2 ml	6 ml	4°C
Buffer B	Detergent and ion chelator mix included.	3 ml	9 ml	4°C/RT **Incubate at RT before use**
Protease Inhibitor mix (P.I. 200x)	200x stock solution	100 µl	300 µl	-20°C
Buffer C	Ion chelator mix included.	4 ml	12 ml	4°C
DNA isolation buffer (DIB)	-	4 ml	10 ml	4°C
Proteinase K	100 x stock solution.	30 µl	90 µl	-20°C
TSH2B primer pairs	5 µM each (Rv & Fw).	50 µl	150 µl	-20°C
c-fos promoter primer pairs	5 µM each (Rv & Fw).	50 µl	150 µl	-20°C
Myoglobin exon 2 primer pairs	5 µM each (Rv & Fw).	50 µl	150 µl	-20°C
PCR tube strips	For 1 row of 8 samples each.	4	12	RT
PCR strip caps	For 1 row of 8 samples each.	4	12	RT

*: LowCell# ChIP kit protein A (Cat. No. kch-maglow-A16/-A48) contains protein A-coated magnetic beads.

**: LowCell# ChIP kit protein G (Cat. No. kch maglow-G16/-G48) contains protein G-coated magnetic beads beads. Please see the troubleshooting guide to understand specificity of proteins A and G.

Table 2: Components available separately

Components available separatly				
Component	**Reference**	**Description**	**Quantity**	**Storage**
DiaMag02 - magnetic rack	kch-816-001	16 positions 0.2ml tubes	1	RT
1 M Sodium butyrate	kch-817-001	-	1 ml	-20°C
Protein A-coated magnetic beads	kch-802-220 kch-802-660 kch-802-150	-	220 µl 660 µl 1500 µl	**4°C** **Do not freeze**
Protein G-coated magnetic beads	kch-818-220 kch-818-660 kch-818-150	-	220 µl 660 µl 1500 µl	4°C
Rabbit IgG	kch-803-015	1 µg/µl	15 µl	4°C
Mouse IgG	kch-819-015	1 µg/µl	15 µl	4°C
Chromatin shearing optimization kit-High SDS	C01020012	includes Buffer B and Glycine	100 million cells	4°C

Other Required Materials Not Provided

Reagents

- Phosphate buffered saline (PBS)
- 1 M Sodium butyrate (NaBu) (Cat. No. kch-817-001)
- Trypsin-EDTA
- Ethanol
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)
- Agarose and TAE buffer
- Formaldehyde (fresh MolBiol Grade)
- qPCR reagents

Equipment and accessories

- DiaMag - magnetic rack (Cat. No. kch-816-001)
- Centrifuge with strip rotor (Galaxy Mini - VWR International, Cat. No. 521-2812)
- Centrifuge for 1.5 ml tubes (4°C)
- Cell counter
- Bioruptor® sonication apparatus (Diagenode Cat. No. UCD-200)
- Rotating wheel (4°C)
- Thermomixer (55°C)

Kit method overview and time table

Table 3: Low Cell# ChIP protocol overview

	Day	Required time
Bind antibody to magnetic beads	1	2 hours
Collect cells and cross-link DNA	1	30 mins + incubation
Lyse cells and shear chromatin	1	1 hour
Perform magnetic IP*	1	2 hours - overnight
Wash immune complexes	1 or 2	30 mins
Purify DNA	1 or 2	1 hour
qPCR and data analysis	1 or 2	2 hours

* The immunoselection can be performed in 2 hours and can result in the same immuno-selection as with an overnight incubation, depending on the antibody used. The use of an ultrasonic water bath can further shorten this protocol -- see the "Additional Protocols" section.

Notes before starting

Cell number

Define the number of cells needed as starting material in function of the number of cells you want to use per IP (see Table 4). You can perform your ChIP with as many as 6 antibodies in one row of 8 tubes or 14 antibodies in two rows of tubes (including one negative and one positive ChIP control per assay). Using more chromatin per ChIP results in more template DNA for PCR analyses so it is advantageous to determine the number of loci one wants to assess by qPCR when determining the cell number to be used for each ChIP. Preparing a common chromatin batch for all IPs ensures that the same cells are analysed for the different markers/modifications in several ChIPs.

Shearing methods and kit compatibility

After cell harvesting, chromatin must be sheared to 200 to 600 bp before ChIP. Our protocols are optimized for use with the Bioruptor® sonication device. It is also possible to use sheared chromatin obtained with our Chromatin shearing optimization kit - High SDS (C01020012) or obtained using another sonication apparatus as long as the buffer composition is adequate and efficient shearing is obtained (see the "Additional Protocols" section).

Shearing optimization and sheared chromatin analysis

You should optimize shearing conditions for your specific cell type and fixation protocol before starting a ChIP. Therefore, start with a small sample (1x 10e5 to 1x 10e6 cells) and check the shearing efficiency. The protocol for shearing analysis is described in "Additional Protocols". Alternatively, see the Chromatin shearing optimization kit - High SDS manual available on www.diagenode.com.

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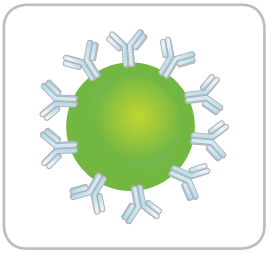
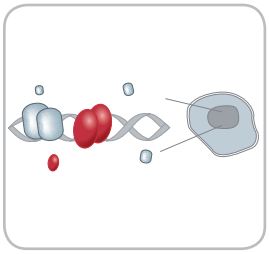
Short Protocol for LowCell# ChIP Kit

STEP 1. Bind the antibodies to magnetic beads



1. Add 22 µl of ice cold Buffer A to 11 µl of beads (per IP) and resuspend. Centrifuge for 5 minutes at 1,300 rpm. Discard supernatant and keep pellet. Repeat this pre-wash once. Then resuspend bead pellet in 11 µl Buffer A per IP.
2. Add 90 µl of Buffer A and 10 µl of the pre-washed Protein A-beads for each IP tube (0.2ml tubes).
3. Add the specific antibody and control antibodies (+ and -). Rotate at 40 rpm for 2 hours at 4°C

STEP 2. Collect cells and cross-link DNA to protein



4. Add inhibitors before cell harvest and mix gently. Harvest, wash, and then count cells.
5. Add PBS (including inhibitors) to cells to a final volume of 500 µl.
6. Add 13.5 µl of 36.5% formaldehyde per 500 µl sample. Vortex gently and incubate 8 minutes at RT.
7. Add 57 µl of 1.25 M glycine to the sample. Vortex gently and incubate 5 minutes at RT.
8. Work on ice from this point onwards
9. Centrifuge at 470 x g for 10 minutes at 4°C. Aspirate the supernatant slowly – leave 30 µl behind
10. Wash cells twice with 0.5 ml ice-cold PBS with inhibitors. Gently vortex and centrifuge at 470 x g for 10 minutes at 4°C.

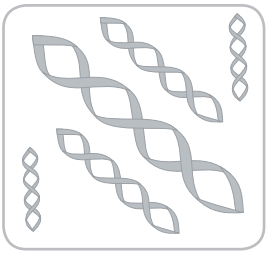
STEP 3. Lyse cells and shear chromatin with Bioruptor®

11. Aspirate the supernatant and leave 10-20 µl behind.
12. Make complete Buffer B by adding protease inhibitor and NaBu. Add 130 µl of complete Buffer B to cells and resuspend.
13. Incubate for 5 minutes on ice. Sonicate for 1 to 3 runs of 5 to 10 cycles (30sec ON / 30 sec OFF)
14. Add 5 µl of Protease Inhibitor mix per ml of Buffer A. Add any desired inhibitors
15. Add 870 µl of complete Buffer A to the 130 µl of sheared chromatin.
16. Assess shearing efficiency

STEP 4. Immunoprecipitate with magnetic rack and wash

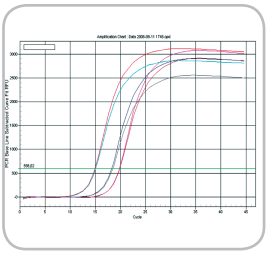
17. Briefly spin the tubes with the antibody-coated beads from step 1.
18. Place tubes in ice cold magnetic rack and wait for 1 minute. Discard supernatant.
19. Transfer 100 µl of diluted sheared chromatin from step 3 to each IP tube in the rack. Keep 10 µl as input at 4°C.
20. Close tube caps. Remove tubes from rack.
21. Incubate on a rotator at 40 rpm for 2 hours up to overnight at 4°C. Ultrasonic bath reduces incubation time.
22. Wash three times using 100 µl of ice cold Buffer A. Discard the buffer after last wash. Keep the captured beads.
23. Wash with 100 µl Buffer C. Capture beads in magnetic rack and remove Buffer C.
24. Centrifuge input samples. Work with both input and IP samples in parallel for remaining steps.

STEP 5. Isolate DNA



25. Add 1 µl of Proteinase K per 100 µl of DNA isolation buffer (DIB) to make complete DIB. Scale as needed.
26. Remove tubes from the magnetic rack and add 100 µl of complete DIB per IP sample. Resuspend the beads and transfer the suspension into 1.5-ml tubes. Add 99 µl of complete DIB to 1 µl of input DNA sample.
27. Incubate all tubes at 55°C for 15 minutes. Then incubate at 100°C for 15 minutes.
28. Centrifuge at 14,000 rpm for 5 minutes at 4°C.
29. Transfer the supernatants into new labeled tubes.

STEP 6. Do quantitative PCR and analyze data



30. Prepare the qPCR mix (total volume of 25 µl/reaction), perform PCR, and analyze.

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Detailed Protocol for LowCell# ChIP Kit

STEP 1. Binding antibodies to magnetic beads

Keep the beads homogenously in suspension at all times when pipetting. Variation in the amount of beads will lead to lower reproducibility.

Note: If you remark that the magnetic bead separation (at any step of this protocol) is not performing properly and some beads are remaining in the bottom of the tube, please proceed as follows:

- Cool down the sample (on ice) for 5 minutes.
- Centrifuge briefly (to spin down droplets that might be in the cap)
- Vortex gently
- Place the tube strip (tubes) again on the magnetic rack to perform the magnetic separation

Note: The LowCell# ChIP kit has been optimized with Diagenode's ChIP-grade antibodies and thus requires low amounts of antibody per IP. The binding capacity of 10 µl of magnetic beads is ~3µg of antibody. If you plan to use more than 3 µg of antibody per IP we recommend that the quantity of beads is adjusted accordingly. Please contact us for advice if required. Protein A coated magnetic beads (C03010020) and Protein G coated magnetic beads (C03010021) are available separately.

1. Each IP requires 11 µl of beads. Add 22 µl Buffer A to 11 µl stock solution of beads for each IP and scale accordingly.
2. Wash the Protein A-coated paramagnetic beads in ice cold Buffer A as follows: suspend beads in Buffer A, centrifuge for 5 minutes at 1,300 rpm, and then discard the supernatant. Keep the bead pellet. Repeat wash one time.
3. Resuspend the pelleted beads in Buffer A to the same concentration as the stock. Add 11 µl of Buffer A for each IP and scale accordingly (e.g. for 2 IPs: add 22 µl of Buffer A).
4. Aliquot 90 µl of Buffer A per PCR tube for each IP. Use the 8-tube strips provided in the kit or individual 0.2 ml tubes.
5. Add 10 µl of pre-washed Protein A-beads to each IP tube.
6. Add the specific antibody and control antibodies (positive and negative) to each tube.
   - Add 1 to 3 µg of antibody or more (up to 10 µg per reaction), depending on the antibody used.
   - Antibody will bind to the beads.
   - See troubleshooting guide for binding capacities of Protein A and G.
7. Incubate the IP tubes at 40 rpm on a rotating wheel for at least 2 hours at 4°C.

STEP 2. Cell collection and DNA-protein cross-linking

Step 2 involves harvesting and fixing the cells to prepare the sheared chromatin. See Table 4 for number of cells needed per IP. Note that another one hundred thousand to one million cells are needed to check shearing efficiency.

We recommend to shear chromatin from 1 million to 100,000 cells in Buffer B with Protease Inhibitor. Afterwards, you dilute (± 10 x) the sheared chromatin in Buffer A with Protease Inhibitors based on the number of cells you want to use in each IP reaction (see table below).

The final dilution volume is 100 µl per IP reaction. Prepare an extra dilution for each sample for use an Input Control.

Table 4:

Number of cells per Shearing	Number of cells per IP: 100,000	50,000	20,000	10,000	5,000	1,000
1 million	Buffer B (130 µl) Buffer A (870 µl) **10 IP's**	Buffer B (130 µl) Buffer A (1,870 µl) **20 IP's**	Buffer B (130 µl) Buffer A (4,870 µl) **50 IP's**	Buffer B (130 µl) Buffer A (9,870 µl) **100 IP's**	-	-
500,000	Buffer B (50 µl) Buffer A (450 µl) **5 IP's**	Buffer B (50 µl) Buffer A (950 µl) **10 IP's**	Buffer B (50 µl) Buffer A (2,450 µl) **25 IP's**	Buffer B (50 µl) Buffer A (4,950 µl) **50 IP's**	Buffer B (130 µl) Buffer A (9,870 µl) **100 IP's**	-
100,000	-	-	Buffer B (50 µl) Buffer A (450 µl) **5 IP's**	Buffer B (50 µl) Buffer A (950 µl) **10 IP's**	Buffer B (50 µl) Buffer A (1,950 µl) **20 IP's**	Buffer B (130 µl) Buffer A (9,870 µl) **100 IP's**

Example: If you shear 1 million cells and if you want to perform your ChIP with chromatin equivalent to 50,000 cells, we recommend to shear your cells in 130 µl **Buffer B** with Protease Inhibitor and to dilute the sheared chromatin with 1,870 µl **Buffer A** with Protease Inhibitor (20 IPs can be performed with this dilution).

8. Prepare and harvest cells as follows:

- Immediately before cell harvesting, if necessary, add any desired inhibitors (eg. HDAC inhibitors such as 20mM NaBu) to the culture medium and mix gently. If adding sodium butyrate* (NaBu) from a 1M stock solution, dilute to reach a final concentration of 20mM. The protocol below includes the use of NaBu-PBS for histone ChIPs. The complete PBS mentioned below refers to PBS with inhibitor(s).

  *** HDAC inhibitor (available separately; Cat. No. kch-817-001).**

- Place PBS at room temperature (RT).
- If using adherent cells, discard medium to remove dead cells. Wash cells by adding 10 ml PBS. Harvest cells by trypsinization using trypsin with inhibitor(s). Transfer cells in a tube containing 10 ml PBS (RT), and centrifuge 5 minutes at 1,300 rpm. Keep the cell pellet and discard the supernatant. Optionally, before transferring, the trypsin can be inactivated by adding serum and inhibitors (if necessary. Then wash the cells in complete PBS and discard medium.
- If using suspension cells, centrifuge for 5 minutes at 1,300 rpm. Keep the cell pellet and discard the supernatant. Wash cells by adding 10 ml PBS (RT) containing 20 mM sodium butyrate (NaBu-PBS) or any other inhibitor.

9. Count the cells (e.g. determine the number of cells in about 200 µl of your sample).

10. Label new 1.5 ml tube(s). Add PBS to a final volume of 500 µl after the cells have been added. If necessary add inhibitors. Then, transfer cells and wash the pipette tip thoroughly in the sample.

    - In order to preserve the cells, use either a 1000 µl pipette tip or a smaller tip that has been cut in order to increase the opening.

11. Add 13.5 µl of 36.5% formaldehyde per 500 µl of sample (final concentration should be ~1%).

12. Mix by gentle vortexing. Incubate for 8 minutes at room temperature to enable fixation. Optimization of fixation time may be required depending cell type, it could be 8-10 minutes.

13. Add 57 µl of 1.25 M Glycine to the sample.

14. Mix by gentle vortexing. Incubate for 5 minutes at room temperature to stop the fixation. Work on ice from this point onwards.

15. Centrifuge at 470 x g for 10 minutes at 4°C.

    - We recommend the use of a swing-out rotor with soft settings for deceleration.

16. Aspirate the supernatant slowly and leave approximately 30 µl of the solution. Do not disturb the pellet. Take care to not remove these cross-linked cells.

17. Wash the cross-linked cells twice with 0.5 ml of ice cold PBS with 20mM final concentration NaBu and/or any other inhibitor of choice.

    - Add the NaBu-PBS solution, gently vortex and centrifuge at 470 x g (in a swing-out rotor with soft settings for deceleration) for 10 minutes at 4 °C.
    - For 100,000 cells or more you might need to resuspend with a pipette to ensure cells are thoroughly washed.
    - Smaller cell numbers are more easily washed and resuspended by vortexing.
    - Make sure that cells are in suspension before proceeding to the next point.

STEP 3. Cell lysis, chromatin shearing

This section describes cell lysis and Bioruptor® chromatin shearing. It is essential to produce fragments of sizes suitable for ChIP and subsequent PCR analysis of the immunoprecipitated DNA. The optimal size range of chromatin for ChIP and the subsequent analysis is between 200 and 600bp.

Place the **Buffer B** at room temperature (RT) before use.
Work on ice unless otherwise stated.

18. After the last wash, aspirate the supernatant, and leave 10 to 20 µl behind to avoid material loss.

19. Prepare Buffer B. Add protease inhibitor (1x final concentration) and if necessary, NaBu (20mM final concentration) to Buffer B (RT). This is the complete Buffer B. Keep the buffer at room temperature until use. Discard what is not used within a day.
Attention: Make sure that there are no crystals in the Buffer B. Gently heat and mix until crystals disappear.

20. Add complete Buffer B to cells. See Table 4 in Step 2 "Cell collection and DNA-protein cross-linking."
Refer to Table 4 for the volume of complete Buffer B to add to cells. Vortex until resuspended. Incubate for 5 minutes on ice.

21. Shear the chromatin by sonication using the Bioruptor®. Shear for 1 to 3 runs of 5 to 10 cycles [30 seconds "ON", 30 seconds "OFF"] at high power setting. Briefly vortex and spin between each run. These shearing conditions will work excellent for many cell types.Optimization is needed depending on the cell type and Bioruptor® system used.

    - See the "Additional Protocols" section for instructions on using other sonication devices.

22. Use the sheared chromatin directly in ChIP.

    - The sheared chromatin can also be stored for subsequent experiments and shearing efficiency analysis (analysis step).
    - For longer term storage, aliquot 130 µl of sheared chromatin into cryotubes, snap-freeze in liquid nitrogen, and store at -80°C. The chromatin can be stored for several weeks or months depending on your ChIP target.
    - Do not freeze/thaw.

23. Prepare Buffer A. Add 5 µl of Protease Inhibitor mix per ml of Buffer A. If necessary, add NaBu (20 mM final concentration for HDAC inhibition) or any other inhibitor to Buffer A. This is the complete Buffer A. 870 µl of complete Buffer A is needed per sample.

24. Add complete Buffer A to cells. See Table 4 in Step 2 "Cell collection and DNA-protein cross-linking" for the volume of complete Buffer A to add to cells. This dilution step reduces the SDS concentration (to ~0.1%) before the addition of antibodies. You may dilute samples further to scale down the number of cells than what is recommended below.

Note: Chromatin from 1,000 cells is sufficient for one ChIP assay (depending on target and antibody used).

25. Assess shearing efficiency before proceeding and use chromatin from 100,000 to 1,000,000 cells to analyze. See "Additional Protocols" section for analysis step.

26. Follow the guidelines in Table 4 to determine the amount of chromatin to be used per IP. Note: Each ml of chromatin is used for 9 IPs + 1 input sample and each 500 µl of chromatin is used for 4 IPs + 1 input sample (see next step).

STEP 4. Magnetic Immunoprecipitation and washes

Step 4 describes the immunoprecipitation of the protein-DNA complex of interest and the washes of the IP'd material.

27. Briefly spin the 0.2 ml tubes containing the antibody-coated beads (from Step 1, point 7) to bring down liquid from the lid.

28. Place tubes in the ice cold magnetic rack (cooled by placing on ice) and wait for 1 minute.

29. Discard the supernatant. Keep the pellet of antibody-coated beads.

30. Add 100 µl of diluted sheared chromatin per IP (see dilution recommendations from table 4) to each 0.2 ml IP tube on the rack. Set aside 10 µl as input sample and keep at 4°C.

31. Close the tube caps and remove tubes from magnetic field.

32. Incubate on a rotator at 40 rpm at 4°C for 2 hours up to overnight.

- See the "Additional Protocols" section to reduce the incubation time using an ultrasonic bath.

33. Place the 8 tubes strip in the magnetic rack, wait 1 minute, and discard the buffer. Wash the beads with 100 µl of ice cold Buffer A. Repeat washes 2 more times . (Note: use 150 µl of Buffer A for each wash if working in a 1.5 ml tube magnetic rack (not provided)). Each wash is done as follows: Add buffer, close the tube caps, invert the 8-tube strip to resuspend the beads, incubate for 4 minutes at 4°C on a rotating wheel (40 rpm), spin, place in the magnetic rack, wait 1 minute, and discard the buffer. Keep the captured beads.

- Do not disturb the captured beads attached to the tube wall.
- Always briefly spin the tubes to bring down liquid in the lid prior to placing in the Diagenode Magnetic Rack.
- Attention: Washes of the beads are critical to isolate the chromatin complexes that are specifically attached to the antibody- coated beads.

34. Wash beads one time with 100 µl of Buffer C. (Note: Use 150 µl of Buffer C for each wash if working in a 1.5 ml tube magnetic rack (not provided)). Each wash is done as follows: Close the tube caps, invert the 8-tube strip to resuspend the beads, and incubate on a rotating wheel (40 rpm) for 4 minutes at 4°C. Spin and place the clean tubes now containing the beads in the magnetic rack after washing. Capture the beads and remove Buffer C. Chromatin complexes specifically bound to the beads have been isolated.

STEP 5. DNA isolation

This kit includes a DNA isolation buffer for easy and fast DNA isolation, resulting in DNA suitable for qPCR analysis. For DNA of higher purity for next generation sequencing or other downstream applications, use the IPure kit (Cat. No. AL-100-0100). Diagenode's IPure kit is the only DNA purification kit that is specifically optimized for extracting very low amounts of DNA after ChIP & MeDIP.

Additional notes:

- Use barrier filter tips, from this step onwards.
- Use the PCR-grade water provided in the kit from this step onwards
- Use the table below to determine whether DIB or IPure is suitable for your particular experiment

Table 5:

	DIB	IPure (Cat. No. AL-100-0100)
Time	1h15	5h40
DNA concentration	+	++ (possible to concentrate)
DNA purity	+	++
Subsequent analysis	qPCR	Next generation sequencing, microarray, qPCR amplification

35. Prepare complete Buffer DIB per sample by adding 1 µl of Proteinase K per 100 µl of Buffer DIB. Scale accordingly and note that 100 µl are needed per IP'd DNA sample and 99 µl per input DNA sample.

36. Remove the tubes from the magnetic rack and add 100 µl of the complete DIB per IP'd DNA sample. Resuspend the beads and transfer the suspension into 1.5-ml tubes.

37. Add 99 µl of complete DIB to 1 µl of input DNA sample.

38. Incubate both the IP'd DNA sample and input DNA sample at 55°C for 15 minutes.

39. Incubate all the samples at 100°C for 15 minutes.

40. Centrifuge at 14,000 rpm for 5 minutes at 4°C.

41. Transfer the supernatants in new 1.5 ml labeled tubes. This is the DNA ready for qPCR analysis. Store at -20°C.

STEP 6. Quantitative PCR & Data analysis

The final step consists of amplifying and analysing the IP'd DNA.

1. Prepare the qPCR mix using SYBR Green master mix.

   qPCR mix (total volume of 25 µl/reaction):

   - 1 µl of provided primer pair (stock: 5 µM each: reverse and forward)
   - 12.5 µl of master mix (e.g.: iQ SYBR Green supermix)
   - 5.0 µl of purified DNA sample and purified input(s)
   - 6.5 µl of water

Table 6:

qPCR cycles			
	Temperature	Time	Cycles
PCR Amplification	95°C	3 minutes	x1
	95°C	30 seconds	x40
	60°C	30 seconds	
	72°C	30 seconds	
Melting Curve	65°C and increment of 0.5°C per cycle	1 minute	x60

2. When the PCR is done, analyse the results. See the appendix for tips on optimizing qPCR.

Appendix

Tips on qPCR

Primer design

- Annealing temperature of 60°C is recommended for qPCR primers.
- Short length of amplified DNA fragment (50 -150 bp) reduces problems in GC-rich region, and results in better PCR efficiency amplification.
- Difference in melting temperature between forward and reverse primers should not exceed 2-3°C.
- GC stretches at the 3' end of the primers should be avoided.
- Primers should amplify unique DNA products from the genome.
- Visit http://frodo. wi.mit.edu/cgi-bin/primer3/primer3_www.cgi to check for good primer design.
- Visit http://www.gene-quantification.info to understand various qPCR technical aspects and analysis methods.
- Visit http://genome.cse.ucsc.edu/cgi-bin/hgPcr to test primer sets. Test every primer set in qPCR using 10 fold-serial dilutions of input DNA. Calculate amplification efficiency (AE) of primer set using the following by formula(5): AE= 10^(-1 / slope)
- The ideal amplification factor is 2. If not, test different qPCR reagents or new primers.

Data Analysis and interpretation

qPCR efficiency

Use a standard curve generated from fragmented genomic DNA from a dilution series of the DNA with desired ChIP primer to determine qPCR efficiency. Most qPCR programs allow automatic calculation of the DNA quantity by comparing Ct and known quantities of DNA standards.

ChIP efficiency

The efficiency of chromatin immunoprecipitation of particular genomic locus is calculated from qPCR data as a percentage of starting material: % (ChIP/ Total input).

% (ChIP/ Total input)= 2^[(Ct(x%input) – log(x%)/log2) -Ct(ChIP)]x 100%

Or

% input= AE^(Ctinput – Ct(ChIP) x Fd x 100%

- Ct (ChIP) and Ct (x%input) are threshold values obtained from exponential phase of qPCR for the IP'd DNA sample and input sample respectively
- (logx%/log2) accounts for the dilution 1:x of the input.
- Fd is a dilution factor of the input DNA to balance the difference in amounts of ChIP and input DNA from qPCR.

Relative occupancy

Relative occupancy is a measure of the protein association with a specific locus and indicates the specificity of ChIP. Highly specific ChIP can result in ~10-1000 fold enrichment over background. This value depends on both the antibody and the target.

Relative occupancy can be calculated as a ratio of specific signal over background:

Occupancy= % input (specific loci) / % input (background loci)

ChIP result can be considered as reliable in case of significant values for both efficiency and specificity.

Sample results with and without a standard curve

1. Use of a standard curve (see additional protocol)

A. standard curve is generated from fragmented genomic DNA. It is recommended to prepare DNA from the same species or the same cell type. Make serial dilutions of the DNA covering the area of concentration of the ChIP samples. Make 8 different concentrations with a broad range. qPCR program allows an automatic calculation of the quantity in the samples by comparing with the Ct and known quantities of the standards.

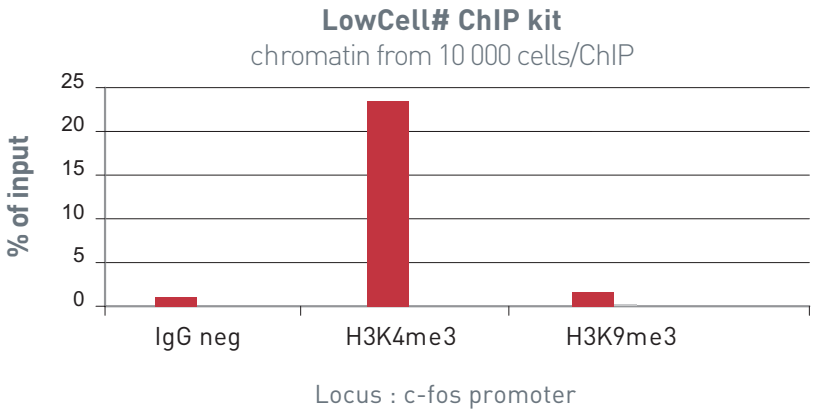
Raw data (standard, Input and IP)		
	Starting Quantity (SQ, ng)	Ct values
Standard 1	2.01 E+00	27.98
Standard 2	1.0 E+00	29.14
Standard 3	5.17 E-01	30.38
Standard 4	2.59 E-01	31.89
Standard 5	1.51 E-01	32.31
Standard 6	1.23 E-02	34.65
Standard 7	2.17E-02	34.75
Standard 8	9.58E-03	35.83

Raw data (standard, Input and IP)			
Identifier	Threshold Cycle (Ct)	Starting Quantity (SQ)	SQ Mean
INPUT 1/100	34.68	1.95E-02	1.25E-01
INPUT 1/100	31.36	2.30E-01	1.25E-01
IgG	33.36	5.20E-02	5.47E-02
IgG	33.23	5.74E-02	5.47E-02
H3K4me3	27.86	3.09E+00	2.92E+00
H3K4me3	28.02	2.75E+00	2.92E+00
H3K9me3	31.26	2.48E-01	2.09E-01
H3K9me3	31.76	1.71E-01	2.09E-01

Calculation: % of input= quantity of the IP/quantity of the INPUT (1%)

IgG	H3K4me3	H3K9me3
0.44	23.36	1.67

Figure A.



With a standard curve

ChIP assays were performed using the LowCell# ChIP kit and undifferentiated human teratocarcinoma (NCCIT) cells, Diagenode antibodies directed against H3K4me3 (Cat. No. pAb-003-024), and against H3K9me3 (Cat. No. pAb-056-050) as well as optimized qPCR primers to amplify a region of the c-fos promoter (Cat. No.: pp-1004-050, -500) from the IP'd DNA were also used. Chromatin was sheared from 100,000 cells (STEP 3). Per ChIP experiment: 10,000 cells equivalent and 1 µg of antibody were used (STEP 4). A negative control antibody was included in the ChIP assay (negative IgG from rabbit: 1 µg/IP). A standard curve was used to present the data.

2. Use without a standard curve (direct Ct values)

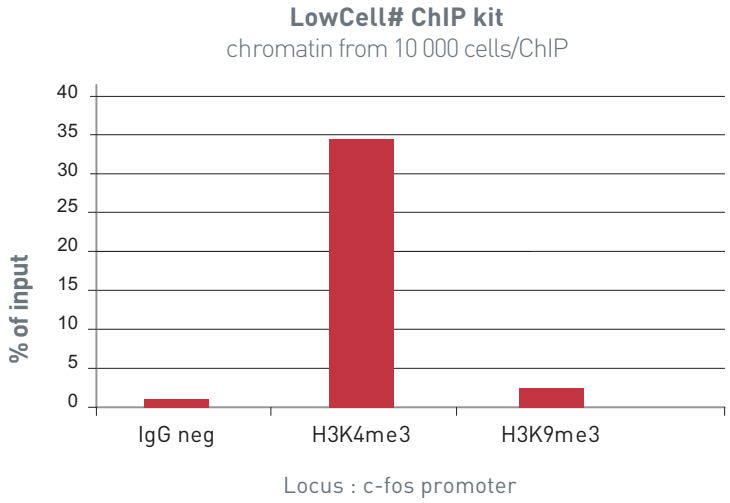
qPCR was performed without a standard curve and the Ct values were reported in the three tables (see raw data below). A compensatory factor (log(100)/log2) was substracted from the Ct values of the diluted input (1 %) in order to calculate the Ct values of the 100% input.

Raw data (Ct values)			
Ct INPUT 1/100 Mean	Compensatory facor	Ct INPUT 100%	Ct IgG Mean
33.0	6.6	26.4	33.3
Ct INPUT 1/100 Mean	Compensatory facor	Ct INPUT 100%	Ct H3K4me3 Mean
33.0	6.6	26.4	27.9
Ct INPUT 1/100 Mean	Compensatory facor	Ct INPUT 100%	Ct H3K9me3 Mean
33.0	6.6	26.4	31.5

Calculation: % (ChIP/ Total input)= 2^[(Ct(x%input) – log(x%)/log2) - Ct(ChIP)]x 100%

IgG	H3K4me3	H3K9me3
0.8	33.8	2.8

Figure B.



Without a standard curve

ChIP assays were performed using the LowCell# ChIP kit and undifferentiated human teratocarcinoma (NCCIT) cells, Diagenode antibodies directed against H3K4me3 (Cat. No.: pAb-003-024) and against H3K9me3 (Cat. No. pAb-056-050) as well as the optimized qPCR primer pair to amplify a region of the c-fos promoter (Cat. No.: pp-1004-050, -500) from the IP'd DNA were also used. Chromatin was sheared from 100,000 cells (STEP 3). Per ChIP experiment: chromatin 10,000 cells equivalent and 1 µg of antibodies were used (STEP 4). A negative control antibody was included in the ChIP assay (negative IgG from rabbit: 1 µg/IP).

Aditional Protocols

Sheared chromatin analysis

This protocol refers to the Diagenode's Elution Module (Cat. No. mc-magme-002) that can be ordered separately.

Reagents not supplied:

- RNase cocktail (e.g. Ambion, AM 2286 A)
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)
- 100% Ethanol
- 70% Ethanol

1. Take an aliquot of 100 µl of sheared chromatin and spin it at 12,000 rpm for 10 min at 4°C. Transfer the supernatant to a new tube for chromatin analysis.
2. Prepare RNase cocktail dilution (e.g. Ambion, AM 2286 A: dilute 1µl of cocktail in 150 µl of water).
3. Add 2 µl of diluted RNase cocktail.
4. Incubate 1h at 37°C.
5. Prepare the Complete Elution Buffer by mixing thoroughly Buffers D, E and F as follows

Reagents	Volume
Buffer D	96 µl
Buffer E	10 µl
Buffer F	4 µl
Total volume	110 µl

6. Add 100 µl of the Complete Elution Buffer to each chromatin sample.
7. Mix thoroughly before incubating sample at 65°C for 4h (or overnight).
8. Extract DNA once with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). Incubate the sample at RT for 10 min on a rotating wheel.
9. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
10. Add 1 volume of chloroform/isoamyl alcohol (24:1). Incubate the sample at RT for 10 min on a rotating wheel.
11. Centrifuge for 2 min at 13,000 rpm at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
12. Precipitate the DNA by adding 5 µl of meDNA coprecipitant, 20 µl of meDNA precipitant and 500 µl of cold 100% ethanol to the sample. Incubate at -80 °C for 30 min.
13. Centrifuge for 25 min at 13,000rpm at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70% ethanol to the pellet.
14. Centrifuge for 10 min at 13,000 rpm at 4°C. Carefully remove the supernatant, leave tubes opened for 30 min at RT to evaporate the remaining ethanol.
15. Re-suspended the pellet in 20 µl of TE buffer.
16. Run samples (20 µl of DNA + 4 µl of 6x loading dye) on a 1.5% agarose gel. As an alternative run 1 µl on a microfluidic chip (e.g. Bioanalyzer High Sensitivity DNA chip).

Reduction of immunoselection time with an Ultrasonic bath

1. Use 100 µl of diluted sheared chromatin per IP (STEP 3, Point 24.). Transfer 100 µl to each 0.2 ml IP tube. Keep 100 µl as Input sample, store at 4°C.
2. Add the specific antibody, control antibodies (positive and negative) and close the tube caps. Vortex, for 5 seconds, at medium power.
   - Add 1 to 3 µg of antibody or more (up to 10 µg per reaction), depending on the antibody used.
   - See troubleshooting guide: for binding capacities of Protein A (and G).
3. Briefly spin the 0.2 ml tubes containing the antibodies to bring down liquid caught in the lid.
4. Incubate for 30 minutes in the ultrasonic water bath. (4°C) ("TsG")
   - Water bath description is in the troubleshooting guide.
   - You might want to optimize the incubation time (see troubleshooting guide).
5. Briefly spin the 0.2 ml tubes to bring down liquid caught in the lid and add 10 µl of pre-washed Protein A-bead per IP tube (STEP 1, Point 2.).
6. Incubate the IP tubes at 40 rpm on a rotating wheel for 1-2 hours at 4°C.
7. Briefly spin the 0.2 ml tubes containing the antibody coated beads to bring down liquid caught in the lid.

From this point on, please folow the instructions of the detailed protocol starting with STEP 4, point 33.

DNA standard curve

Run samples of known DNA concentrations to get a standard curve for each qPCR plate run. Make a series of dilutions (up to 8) of one unmanipulated, fresh gDNA sample. This calibration allows you to quantify the relative amount of DNA.

- Prepare DNA from the same species/same cell type as was used in the ChIP.
- Prepare chromatin from the cells in the same manner as for ChIP.

An example is given below. Using 100,000 NCCIT cells to start with, in 50 µl of TE, we got 3.02 ng of DNA /µl (or 15.1 ng/ 5 µl). The DNA sample was then diluted as shown below and 5 µl were used per qPCR well:

Standard 1	dilution 1/2 → 7.55 ng/ 5 µl
Standard 2	dilution 1/5 → 3.02 ng/ 5 µl
Standard 3	dilution 1/10 → 1.51 ng/ 5 µl
Standard 4	dilution 1/50 → 0.302 ng/ 5 µl
Standard 5	dilution 1/200 → 0.0755 ng/ 5 µl
Standard 6	dilution 1/500 → 0.0302 ng/ 5 µl
Standard 7	dilution 1/1000 → 0.0151 ng/ 5 µl
Standard 8	0 ng

Troubleshooting guide

Process	Protocol step	Issues and resolutions
Crosslinking and fixation	Optimize crosslinking time	Poor crosslinking causes DNA loss, elevated background, and/or reduced antigen availability in chromatin. Emperically determine optimal crosslinking time for maximal specificity and efficiency of ChIP. The optimal duration of cross-linking varies between cell type and protein of interest. Short crosslinking time (5-10 minutes) may improve shearing efficiency. Crosslinking duration should not exceed 30 minutes or shearing will be inefficient.
	Assure proper fixation time with formaldehyde	Crosslinking may be too weak or too strong without proper fixation time. Optimize fixation step e.g: incubate for 8 minutes at room temperature with high-quality, fresh 1% formaldehyde final concentration (weight/volume).
	Optimize formaldehyde concentration	Lower formaldehyde concentrations (1%weight/volume) may improve shearing efficiency. For some proteins, however, especially those that do not directly bind DNA, this might reduce crosslinking efficiency and thus the yield of precipitated chromatin. Empirically determine the formaldehyde concentration as some antigen epitopes may be more sensitive to formaldehyde.
Cell lysis	Make sure cells disrupt completely	Do not use too many cells per amount of lysis buffer (w/v) so that cells can be completely disrupted. Follow the instructions in the protocol (e.g.: 1 million cells or less/130 µl of complete Buffer B). See steps 2 and 3.
	Maintain cold temperature during lysis	Perform cell lysis at 4°C (cold room) or on ice. Always keep the samples ice cold during cell lysis and use cold buffers as in Step 3.
	Prevent protein degradation	Add the protease inhibitors to the lysis buffer immediately before use.
Cell type	Determine which cell types have previously been validated with the kit	HeLa, NCCIT 293T, Chondrocytes, P19, ASC (adipose stem cells), U2OS and kerationocytes have been used to validate this magnetic ChIP protocol.
Number of cells required	Determine number of cells for ChIP	The number of cells for ChIP is determined by cell type, protein of interest, and antibodies used. Use chromatin from 1,000 to 10,000 cells per ChIP. (In some cases, chromatin from up to 100,000 cells may be needed). You may need to empirically determine the optimal number.
Chromatin shearing	Maintain 4°C temperature during shearing	Keep samples cold at 4°C before sonication to maintain sample integrity.
	Maintain 0ÐC temperature during sonication	Maintain temperature of the samples at 4°C to maintain sample integrity.
	Optimize SDS concentration	High % SDS favours better sonication but inhibits immunoselection (optimal range: 0.1% to 1%). 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 ChIP buffer.
	Determine amount of sheared chromatin needed for ChIP	Most of the sheared chromatin will be used for ChIP and the input control. A small amount will be checked on agarose gel.
	Dilute the sheared chromatin in ChIP buffer for Immunoselection incubation	The sheared chromatin is diluted in complete Buffer A prior to the immunoselection incubation (see Step 3). Dilute the sheared chromatin at least 7-fold. Adjust the ChIP buffer volume added to the chromatin accordingly.

Sonication tips	Determine cell number	Start with 1x10e6 cells or less.
	Sonication conditions with the Bioruptor tips	Shear the samples of chromatin using the Bioruptor® for 10-12 cycles of: [30 seconds "ON" / 30 seconds "OFF"] each. These conditions were tested with many mammalian cell lines and were excellent for subsequent ChIP experiments. A troubleshooting guide for Bioruptor-chromatin shearing is available.
	Chromatin shearing with a probe sonicator tips	Probe sonicator: Sonicate each sample for 3 x 30 seconds on ice. Allow 30 seconds pause on ice between each pulsing session. Avoid foaming.
	Chromatin shearing with Diagenode modules tips	You can also use the Transcription ChIP kit (Cat. No. kch-redmod-100 or -400) for shearing. 30 µl of shearing buffer is added per million cells. After shearing, transfer the needed amount of chromatin to a new tube and add Buffer B from the LowCell# ChIP kit to reach130 µl.
	Shearing with other protocols tips	When using your own protocol,make sure the shearing buffer contains between 0.75% and 1% SDS, EDTA (1-10 mM) and/or EGTA (0-0.5 mM) with pH 7.6-8.0. The sheared chromatin is to be diluted in the Buffer A and Buffer B prior to immunoselection.
Gel analysis of sheared chromatin	Load enough DNA on gel	Chromatin equivalent to at least 100,000 cells can be be visualized on a gel. Do not use an excessive amount or it will obscure the visualization. The DNA amount to load depends on well size and on the gel size.
	Use correct agarose concentration	Use a 1-1.5% agarose gel.
	Use correct running buffer concentration and run time	1x TAE or TBE is preferred to 0.5x TAE (which can lead to smears). Run slowly.
Antibody-bead binding	Make sure beads are in suspension.	The provided beads are coated with protein A. Resuspend into a uniform suspension before each use.
	Use proper bead centrifugation methods	Use gentle centrifugation (500 x g for 2-3 minutes) as described in the manual protocol. g= (1.118 x 10-5) x r x rpm2 where r is the radius (www.msu.edu/~venkata1/gforce.htm). It is possible to centrifuge the 1.5 ml tubes at 1,000 – 2,000 g for 20 seconds.
	Store beads at 4°C	Store at 4°C. Do not freeze.
	Determine antibody binding capacity	pAb from rabbit, guinea pig, pig, human IgG. MAb from mouse (IgG2), human (IgG1,2 and 4); and rat (IgG2c).
Protease inhibitors and other inhibitors	Store protease inhibitors properly	Some inhibitors are unstable in solution. The provided P.I. mix should be kept frozen at -20°C and thawed before use.
	Always use fresh, complete buffers	Add protease inhibitor mix to buffers, just before use, in PBS (Steps 2 and 3), Buffer B (Step 3), in Buffer A (Step 3). Discard within 24 hours.
	Use other inhibitors as needed.	Add phosphatase inhibitors or others to Buffers A and B, if necessary, depending on your research field and protein(s) of interest. Add NaBu for histone ChIPs.
Negative ChIP controls	Use non-immune IgG in the IP incubation mix.	Use the non-immune IgG fraction from the same species the antibodies were produced in as a negative control.
	Do not add antibody to the IP to serve as a negative control	Incubation with uncoated beads could also be used as a negative ChIP control (see Step 4).
	Use an unblocked antibody and specifically blocked antibody in parallel	Use one antibody in ChIP and the same antibody that is blocked with specific peptide. To specifically block 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.
	Determine number of negative controls needed	If multiple antibodies of the same species are to be used with the same chromatin preparation then a single negative ChIP control is sufficient for all of the antibodies used.

<table>
<tr><td rowspan="4">Antibody in IP</td><td>Antibody-antigen recognition may affect ChIP</td><td>Antibody-antigen recognition can be significantly affected by crosslinking resulting in loss of epitope accessibility and/or recognition.</td></tr>
<tr><td>Use ChIP-grade antibodies, include controls, and test antibodies before ChIP</td><td>Use ChIP-grade antibodies or several antibodies directed against different epitopes of the same protein. Verify that the antibodies work directly in IP on fresh cell extracts. When testing new antibodies, include known ChIP-grade antibodies as a positive control. Be aware of the possible cross-reactivity of antibodies. Verify by Western blot analysis the antibody specificity. Antigen affinity purification can be used to increase titer and specificity of polyclonal antibodies.</td></tr>
<tr><td>Determine amount of antibody per ChIP</td><td>Empirically determine amount of target and antibody. For abundant proteins, like histones, use 1-2 µg of affinity purified or monoclonal antibody per IP. For other targets, use up to 10 µg per ChIP. Efficient IPs result from optimal ratios between the amount of chromatin and the amount of antibody. More antibody (or less chromatin) can be required with low affinity to antigen or high abundance of target protein (e.g. histones). Insufficient amount of antibody can result in low efficiency of ChIP whereas large excess of antibody might lead to lower specificity.</td></tr>
<tr><td>Concerns about antibodies binding to protein A or protein G.</td><td>There is a significant difference in affinity of different types of immunoglobulins to protein A or G. Thererfore, in function of the antibody used for your ChIP, it is recommended to choose either protein A or protein G coated beads.
<table>
<tr><th>Species</th><th>Immunglobulli Isotype</th><th>Protein A</th><th>Protein G</th></tr>
<tr><td>Human</td><td>IgG1</td><td>+++</td><td>+++</td></tr>
<tr><td></td><td>IgG2</td><td>+++</td><td>+++</td></tr>
<tr><td></td><td>IgG3</td><td>-</td><td>+++</td></tr>
<tr><td></td><td>IgG4</td><td>+++</td><td>+++</td></tr>
<tr><td></td><td>IgGM</td><td colspan="2">Use anti Human IgM</td></tr>
<tr><td></td><td>IgGF</td><td>-</td><td>+</td></tr>
<tr><td></td><td>IgGA</td><td>-</td><td>+</td></tr>
<tr><td>Mouse</td><td>IgG1</td><td>+</td><td>+++</td></tr>
<tr><td></td><td>IgG2a</td><td>+++</td><td>+++</td></tr>
<tr><td></td><td>IgG2b</td><td>++</td><td>++</td></tr>
<tr><td></td><td>IgG3</td><td>+</td><td>+</td></tr>
<tr><td></td><td>IgGM</td><td colspan="2">Use anti Mouse IgM</td></tr>
<tr><td>Rat</td><td>IgG1</td><td>-</td><td>+</td></tr>
<tr><td></td><td>IgG2a</td><td>-</td><td>+++</td></tr>
<tr><td></td><td>IgG2b</td><td>-</td><td>++</td></tr>
<tr><td></td><td>IgG2c</td><td>+</td><td>++</td></tr>
<tr><td colspan="2">Chicken All Isotypes</td><td>-</td><td>++</td></tr>
<tr><td colspan="2">Cow All Isotypes</td><td>++</td><td>+++</td></tr>
<tr><td colspan="2">Goat All Isotypes</td><td>-</td><td>++</td></tr>
<tr><td colspan="2">Guinea Pig All Isotypes</td><td>+++</td><td>++</td></tr>
<tr><td colspan="2">Hamster All Isotypes</td><td>+</td><td>++</td></tr>
<tr><td colspan="2">Horse All Isotypes</td><td>++</td><td>+++</td></tr>
<tr><td colspan="2">Pig All Isotypes</td><td>+</td><td>++</td></tr>
<tr><td colspan="2">Rabbit All Isotypes</td><td>+++</td><td>++</td></tr>
<tr><td colspan="2">Sheep All Isotypes</td><td>-</td><td>++</td></tr>
</table></td></tr>
</table>

Immunoselection incubation	Understanding the benefits of using an ultrasonic water bath	The use of ultrasonic energy to enhance mass transport across liquid/solid interfaces can dramatically accelerate antigen binding to antibodies, the typical rate-limiting step in ChIP.
	Buying an ultrasonic bath	See http://www.bransonic.com/model_3510.asp: Branson Cat. No. CPN-952316 or Fisher Scientific Cat. No.15-337-22F.
	Determine water bath specifications	Model MT-3510. Capacity: 5.5 liters. Size (LxWxH): 29x15x15 cm. Frequency: 42 kHz. Max power requirement: 130 W. RF-Power: 130 W
	Optimize the incubation time with an ultrasonic bath	Incubation of 15-30 minutes is usually sufficient but may differ depending on antibody-target kinetics. A longer incubation may be required in some cases.
	Using the kit without an ultrasonic water bath	Without the bath, a long incubation at 4°C should be used. Depending on the antibody and target, the times of incubation range from 2 to 16 hours and should be determined empirically for each antibody.
PCR tips	Optimize primer design	Primer length: 18 to 24 nucleotides and primer Tm: 60°C (+/-3.0°C)/ % GC: 50% (+/-4%)
	Include negative and positive controls	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.
	Troubleshoot high Ct values	Use more input chromatin in the case of high Ct values.
	Determine the ratio between Ct(NegCtl) and Ct(Target)	The ratio between target IP and negative control IP depends on the antibody used.
	Minimize high background	Keep the antibody binding beads in suspension during the experiment. Check by eye that equal pellets of beads are present in each tube. Washes (step 4) are critical.
	Using end-point PCR analysis rather than quantitative PCR	If gel electrophoresis is used to estimate intensities of PCR products, the 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.
Sample storage and freezing	Samples can be frozen at several steps of the protocol	Snap freeze and thaw on ice (e.g. fixed cell pellets and sheared chromatin). 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. Purified DNA from ChIP and input samples can be stored at -20°C for months. Avoid multiple freeze/thawing.

Ordering information

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For a complete listing of Diagenode's international distributors visit:
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