PRODUCT INFORMATION & MANUAL

QuikChIP™
Chromatin Immunoprecipitation Kit

NBP2-29902

Research use only. Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 -
technical@novusbio.com

TABLE OF CONTENTS

I. OVERVIEW

Chromatin Immunoprecipitation (ChIP) is a powerful method used to identify regions of the genome associated with specific proteins. These associations are crucial for vital cellular functions including gene transcription, DNA replication and recombination, repair, segregation, chromosomal stability, cell cycle progression, and epigenetic silencing.[1] The ChIP assay has been used to study both histones and non-histone proteins, such as transcription factors, within the context of the cell.[2,3,4,5] Transcription factors and other DNA binding proteins have a weaker affinity than histones, which generally are tightly associated within the chromatin complex. To avoid dissociation of non-histone proteins from the chromatin binding site, it is necessary to incorporate a cross-link step. Novus' QuikChIP™ Kit has been optimized for use with both histone and non-histone proteins.

Using the QuikChIP™ Assay, cells are fixed with formaldehyde to cross-link nonhistone proteins to DNA. Alternatively, the cross-linking steps are not needed for histone proteins, which are tightly bound to DNA. The chromatin are released from the nuclei and sheared into 200-1000 bp fragments. The chromatin is then immunoprecipitated with specific antibodies. DNA sequences cross-linked to the protein of interest co-precipitate as part of the chromatin complex. The DNA/Chromatin/Antibody complex is then isolated using Protein G Agarose. After reverse cross-linking, the associated DNA is released from the complex and is ready for analysis.

Quality Control Results

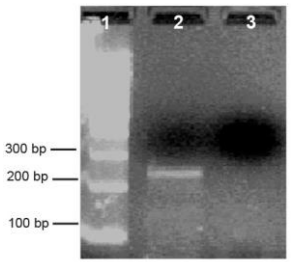
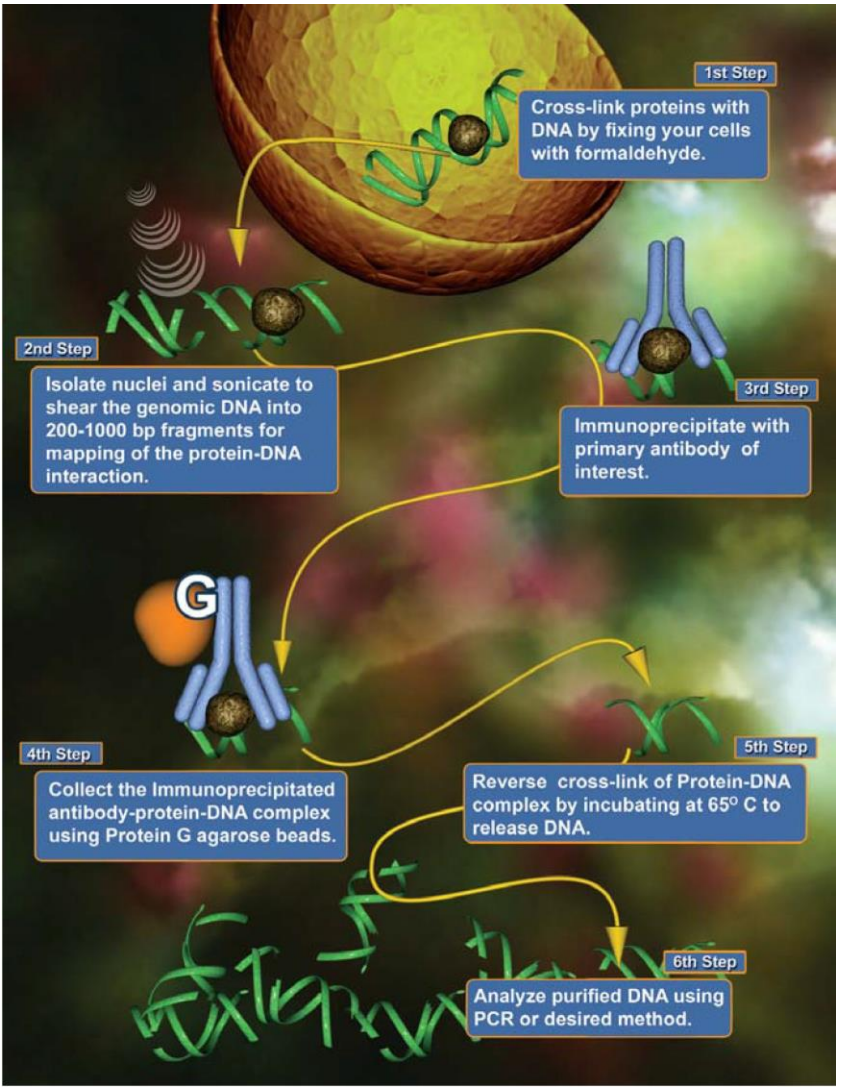


FIGURE 1
Lane 1: 100 bp ladder.
Lane 2: DNMT3b.
Lane 3: No antibody Control.

PCR analysis of immunoprecipitated DNA generated using NOVUS' QuikChIP™ Kit.

Human MCF-7 cells were fixed for 10 minutes with 1% formaldehyde. The cells were lysed and the chromatin sonicated (200-1000 bp fragments). Chromatin was immunoprecipitated using DNMT3b mAb, NOVUS Cat # NB100-56514 (Lane 2) and a no antibody control (Lane 3). The PCR amplification was performed, with primers specific to the progesterone receptor (PR) promoter region, as follows: 94°C 5 min, 94°C 30 sec, 59°C 30 sec 40 cycles, 72°C 30 sec, 72°C 10 min. Following PCR, 20 µl of each sample was analyzed on a 2% agarose gel and visualized by UV-illumination following ethidium bromide staining. PCR product was observed in the DNMT3b mAb ChIP (Lane 2) and not in the control (Lane 3).

FIGURE 2. Overview of the ChIP Assay



II. QuikChIP™ KIT DESCRIPTION

Storage: The QuikChIP™ Kit is shipped on blue ice. Once received, the kit should be opened, and the individual components stored appropriately. Storage conditions are also listed on page seven of this manual. When stored appropriately, the components are stable for one year.

Usage: The QuikChIP™ Kit contains components for 25 ChIP assays optimized for use with transcriptionally active chromatin in adherent or suspension mammalian cells. The QuikChIP™ Kit also contains the buffers suitable for making 25 preparations of sheared chromatin for ChIP. This protocol is optimized for use with one 10 cm plate containing 5 x 10^6 cells per preparation. Each ChIP assay requires approximately 1 x 10^6 cells. Thus, a 10 cm plate (one preparation) of cells will provide enough DNA for five ChIP assays.

Safety Warnings and Precautions: Formaldehyde, phenylmethyl sulfonyl fluoride (PMSF) and protease inhibitor cocktail (PIC) are defined as hazardous (please see MSDS). Formaldehyde should be used in a ventilated fume hood. If tissue culture plates containing formaldehyde are put into a humidified incubator, they should be placed in sealed bags to prevent damaging other cells in the incubator. All chemicals should be handled with the principles of good laboratory practice. Protective ear equipment must be used during sonication.

QuikChIP™ Products from Novus Biologicals

QuikChIP™ Chromatin Immunoprecipitation (NBP2-29902)
QuikChIP™ Chromatin Immunoprecipitation with controls (NBP2-29497)
QuikChIP™ Sheared Chromatin
DNMT1 mAb (NB100-56519)
DNMT3a mAb (NB120-13888)
DNMT3b mAb (NB100-56514)
HDAC3 pAb (NB100-1669)
HDAC4 pAb (NB100-56341)
HDAC5 pAb (NBP2-03988)
HDAC6 pAb (NB100-56343)
p53 pAb (NBP2-27576)
TRF2 pAb (NB100-56694)

III. QUIKChIP™ KIT COMPONENTS

DESCRIPTION	QUANTITY	STORAGE (°C)
Wash Buffer A	25 mL	4
Wash Buffer B	25 mL	4
Wash Buffer C	25 mL	4
Wash Buffer D	50 mL	4
SDS Lysis Buffer	25 mL	4
10X Glycine	25 mL	4
10X PBS	55 mL	4
ChIP Dilution Buffer	50 mL	4
Salmon Sperm DNA / Protein A/G Agarose	2 x 1.75 mL	4
RNase A	10 mg/mL, 60 µL	-20
100X PMSF	2 x 0.75 mL	-20
100X PIC	2 x 0.75 mL	-20
Proteinase K (10 mg/mL)	60 µL	-20
20% SDS	625 µL	RT
1M $NaHCO_3$	1.25 mL	RT
0.5M EDTA	250 µL	RT
5M NaCl	500 µL	RT
1M Tris-HCl, pH 6.5	500 µL	RT

Additional Materials Required (not included in this kit)

Reagents

Cells of interest
Antibody of interest
Appropriate antibody control
37% Formaldehyde
3M Sodium Acetate, pH 5.2
Phenol/chloroform (optional)
100% ethanol
70% ethanol
Taq DNA polymerase
dNTP mixture
10X PCR Buffer
Cell culture media
ddH_2O

Equipment

Agarose gel electrophoresis apparatus
Rotating platform
Microcentrifuge
Microcentrifuge tubes
Spectrophotometer
Variable volume pipettors
Pipette tips
Thermocycler
Thermocycling tubes
Cell Scraper
Aspirator
Sonicator

IV. CHROMATIN IMMUNOPRECIPITATION (IP) PROTOCOL

To perform ChIP, it is necessary to shear chromatin into 200-1,000 bp fragments. The following protocol assumes that optimized shearing conditions have already been determined. If they have not, please proceed to Appendix A (Shearing Optimization Protocol) for a complete protocol. We have successfully used this protocol with mammalian cells including HeLa, 293HEK, Raw, NIH 3T3, MCF7, and Ramos cells.

DAY 1

Before You Begin

- 5 x10^6 mammalian cells on a 10 cm tissue culture plate. Cells should be treated as necessary to insure the transcriptional activation of the gene of interest.
- 1% Formaldehyde Solution at RT. Add 270 µL 37% formaldehyde to 10 mL cell culture media. It is important to use high quality formaldehyde. (Example: Fisher cat. no F79-500)
- 10X Glycine at RT.
- Ice for incubation of cells and PBS. During the procedure, high temperatures will reverse the formaldehyde cross-linking. Care must be taken at each step to keep the cells and reagents on ice whenever indicated in the protocol.
- 1X PBS: Add 3 mL of 10X PBS to 27 mL ddH_2O.
- SDS Lysis Buffer at RT.
- 100X Protease Inhibitor Cocktail (PIC) on ice.
- 100X PMSF on ice.
- Optimize conditions to shear your cross-linked DNA ~200-1,000 bp.

CROSS-LINK

NOTE: Cross-linking procedure may be omitted when using antibodies to Histone proteins which are tightly bound to DNA. Protein properties must be determined empirically.

A. ADHERENT CELLS

1. For adherent cells, carefully aspirate cell culture media from the 10 cm plate containing cells.
2. Add 10 mL of 1% Formaldehyde Solution to plate. This step serves to fix the cells, binding the protein/DNA complex in the nucleus.
3. Incubate at 37°C for 10 min.
4. Add 1 ml of 10X Glycine to plate and gently swirl to mix. This step serves to stop the fixing process, to ensure chromatin do not over fix.
5. Incubate at RT for 5 min.

6. Aspirate medium. Remove as much medium as possible without disturbing cells.
7. Wash cells 2x with 10 mL of ice cold 1X PBS.
8. Add 1 mL ice cold 1X PBS (supplemented with 10 µL 100X PMSF and 10 µL 100X PIC).
9. Scrape cells into a centrifuge tube.
10. Centrifuge at 1,200 rpm (3,000 x g) at 4°C for 5 min.
11. Carefully discard supernatant, leaving behind pelleted cells. Cell pellet can be stored at -80°C for several months.

B. SUSPENSION CELLS

1. For suspension cells, centrifuge cells at 1,200 rpm for 5 min and carefully aspirate cell culture media.
2. Add 10 mL of 1% Formaldehyde Solution to the tube. This step serves to fix the cells, binding the protein/DNA complex in the nucleus.
3. Incubate at 37°C for 10 min.
4. Add 1 ml of 10X Glycine to the tube and gently swirl to mix. This step serves to stop the fixing process, to ensure chromatin does not over fix.
5. Incubate at RT for 5 min.
6. Centrifuge at 1,200 rpm for 5 min. Remove as much medium as possible without disturbing the cell pellet.
7. Wash cells 2x with 10 mL of ice cold 1X PBS.
8. Add 1 mL ice cold PBS (supplemented with 10 µL 100X PMSF and 10 µL 100X PIC).
9. Centrifuge at 12,000 rpm (7,000 x g) at 4°C for 5 min.
10. Carefully discard supernatant, leaving behind pelleted cells. Cell pellet can be stored at -80°C for several months.
11. Continue with Lyse Protocol.

LYSE

12. Resuspend pellet in 1 mL SDS Lysis Buffer (supplemented with 10 µL 100X PMSF and 10 µL 100X PIC).
13. Incubate on ice for 10 min. Optional: remove one 5 µL aliquot of cell lysate for agarose gel analysis of unsheared DNA.

SONICATE

14. Sonicate cell lysate on ice according to optimized conditions determined in Appendix A. Keep cell lysate on ice during sonication.
15. Centrifuge at 15,000 rpm (12,000 x g) at 4°C for 10 min to pellet the insoluble material. Optional: remove one 25 µL aliquot to determine DNA concentration and shearing efficiency.
16. Transfer supernatant to a fresh centrifuge tube. You should have about 1 mL of supernatant, enough for approximately five IPs (200 µL per IP). Sheared sonicated material can be stored at -80°C for several months.

PRECLEAR

Before You Begin

- 100X PIC on ice.
- Determine number of IPs (remember to include an appropriate antibody control).
- Each IP requires that the supernatant be diluted 5 to 10-fold with ChIP Dilution Buffer containing PIC. For example: One IP requires 200 µL supernatant diluted with 790 µL ChIP Dilution Buffer and 10 µL PIC.

17. For each IP you wish to run, aliquot 800 µL ChIP Dilution Buffer containing PIC.
18. Add 200 µL of the supernatant (from Step 16) to each tube.
19. Add 75 µL Salmon Sperm DNA/Protein A/G Agarose to each tube. This step serves to remove molecules that can non-specifically bind to the Protein G Agarose.
20. Rotate for 30 min at 4°C.
21. Briefly centrifuge Salmon Sperm DNA/Protein A/G Agarose at 1,200 rpm (3,000 x g) for 1 min. It is not necessary to centrifuge Protein A/G Agarose beads at high g forces. If you are having trouble pelleting, please see the FAQ section.
22. Collect the supernatant by aliquoting into a fresh microfuge tube.
23. Aliquot a 10 µL sample of the supernatant into a fresh microfuge tube and store at 4°C for use in Step 31 below. This "Input" sample should be used as negative antibody control during PCR analysis on Day 3.

IMMUNOPRECIPITATE

Before You Begin

- Immunoprecipitating antibody (user-provided): The amount of antibody will vary and needs to be empirically determined.

24. Add the immunoprecipitating antibody to the supernatant fraction. For user-provided antibody, the appropriate amount of antibody (usually between 1-10 µg per tube) needs to be determined empirically. For the positive control, add 1 µg of RNA Polymerase II antibody per tube.
25. Rotate overnight at 4°C. Depending on the empirical properties of your antibody and target gene, this step may be shortened.

DAY 2

Before You Begin

- 100X PIC and 100X PMSF on ice (optional addition to wash buffers, user determined).
- 1M $NaHCO_3$ at RT.
- 65°C water bath.
- Prepare 500 µL Elution Buffer (1% SDS, 0.1M $NaHCO_3$) per sample (and for Input tube from Step 23) as follows: 25 µL 20% SDS, 50 µL 1M $NaHCO_3$ and 425 µL ddH_2O).
- Wash Buffer A, B, C and D on ice.

COLLECT

26. Add 60 µL of Salmon Sperm DNA/Protein A/G Agarose for 1 hr at 4°C with rotation. This step serves to collect the Antibody/Antigen/DNA complex.
27. Pellet Protein A/G Agarose by brief centrifugation at 1,200 rpm (3,000 x g) for 1 min and discard the supernatant fraction.

WASH

28. Wash the Protein A/G Agarose/Antibody/Chromatin complex on a rotating platform as follows:
    - Resuspend the beads in 1 ml cold Wash Buffer A.
    - Incubate for 3-5 min on a rotating platform.
    - Briefly centrifuge at 1,200 rpm (3,000 x g) for 1 min and discard the supernatant fraction.
    - Repeat wash with 1 ml cold Wash Buffer B.
    - Repeat wash with 1 ml cold Wash Buffer C.
    - Repeat wash 2x with 1 ml cold Wash Buffer D.

ELUTE

29. Add 250 µL of the Elution Buffer to each Protein A/G Agarose/Antibody/Chromatin complex.
30. Add 500 µL of the Elution Buffer to the Input tube (from Step 23) and set aside at RT until Step 35.
31. Mix by gently flicking tubes.
32. Incubate at RT for 15 min.

33. Pellet the Protein A/G Agarose/Antibody/Chromatin complex by brief centrifugation at 1,200 rpm (3,000 x g) for 1 min and collect supernatant.
34. Repeat the elution steps for the Protein A/G Agarose/Antibody/Chromatin complex a second time and combine eluates (for a total volume of about 500 µL).

REVERSE CROSS-LINK*

35. Add 20 µL 5M NaCl and incubate at 65°C for 4 hr to overnight. This step serves to reverse the DNA/Protein cross-links.
36. Centrifuge for 30 sec to collect liquid from sides of tubes and then add 1 µL of RNase A and incubate for 30 min at 37°C.
37. Add 10 µL 0.5M EDTA, 20 µL 1M Tris-HCl, pH 6.5, 2 µL 10 mg/mL Proteinase K and incubate for 1 hr at 45°C.

** Reverse cross-link step is not necessary if anti-histone antibodies are used to precipitate chromatin.*

DAY 3

DNA PURIFICATION

38. Purify DNA for analysis using the Phenol/Chloroform Extraction Protocol detailed in Appendix B. Alternatively, DNA purification columns can be used for purification.

PCR ANALYSIS AND PCR OF CONTROLS

Accurate PCR analysis of ChIP DNA requires that the PCR is stopped during the linear amplification phase. The appropriate number of PCR cycles for each experiment must be determined empirically.

V. REFERENCES

a. Das PM, K Ramachandran, J van Wert, R Singal. Chromatin immunoprecipitation assay. 2004. Biotechniques 6:961-9.

b. Wells J, PF Farnham. Characterizing transcription factor binding sites using formaldehyde crosslinking and immunoprecipitation. 2002. Methods 1:48-56.

c. Weinmann AS, PJ Farnham. Identification of unknown target genes of human transcription factors using chromatin immunoprecipitation. 2002. Methods 26:37-47.

d. Weinmann, AS. Novel ChIP-based strategies to uncover transcription factor target genes in the immune system. Nat Rev Immunol. 2004. 5:381-386.

e. Kuo MH, CD Allis. In vivo cross-linking and immunoprecipitation for studying dynamic protein:DNA associations in a chromatin environment. 1999. Methods 3:425-33.

f. Leu YW, PS Yan, M Fan, VX Jin, JC Liu, EM Curran, WV Welshons, SH Wei, RV Davuluri, C Plass, KP Nephew, TH Huang. Loss of estrogen receptor signaling triggers epigenetic silencing of downstream targets in breast cancer. Cancer Res. 2004. 22:8184-8192.

VI. APPENDICES

Appendix A: Shearing Optimization Protocol

Before performing ChIP, you must optimize sonication conditions to shear your DNA (~200-1,000 bp). To shorten the protocol, we recommend that you simultaneously prepare cells for both the ChIP protocol and the shearing optimization below. In doing so, once you have optimized you can proceed to the sonication stage at Step 14 on Day One in the ChIP protocol.

CROSS-LINK & LYSIS:

Follow Steps 1-12 from pages 8-9.

13. Remove one 5 µL aliquot of cell lysate for agarose gel analysis of unsheared DNA.
14. Aliquot the cell lysate equally into three centrifuge tubes.

OPTIMIZE SONICATION

15. Shear the three aliquots using three different conditions (Figure 3). For example:
    a. Five pulses of 20 sec each, with a 30 sec rest on ice between each pulse.
    b. Ten pulses of 20 sec each, with a 30 sec rest on ice between each pulse.
    c. Twenty pulses of 20 each, with a 30 sec rest on ice between each pulse.
16. Centrifuge the three sheared chromatin samples at 15,000 rpm (12,000 x g) at 4°C for 10 min.
17. Transfer supernatant to a fresh tube avoiding any pelleted material.
18. Transfer 100 µL of each sheared chromatin to a fresh centrifuge tube.
19. Add 4 µL of 5M NaCl to the sheared chromatin and briefly vortex to mix.
20. Incubate at 65°C for 4 hr to overnight. This step serves to reverse the DNA/Protein cross-links.
21. Centrifuge for 30 sec to collect liquid from sides of tubes.
22. The DNA is ready to run on a 1% agarose gel to determine the size of the sheared DNA. Load differing amounts of each sample (for example: 5, 10, 20 µL of each sample) to avoid overloading.
23. Observe which shearing conditions result in a smear of DNA between 200 and 1,000 bp (see Figure 3).

24. If the DNA smear is not in the desired size range, repeat optimization altering sonication conditions until desired range is obtained.

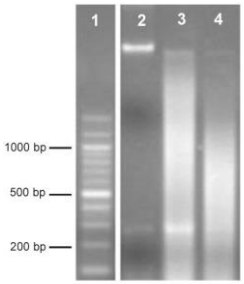


FIGURE 3
Lane 1: 100 to 1000 bp ladder.
Lane 2: Unsheared MCF7 DNA.
Lane 3: MCF7 DNA sheared 5 pulses.
Lane 4: MCF7 DNA sheared 15 pulses.

Analysis of DNA shearing using Novus' QuikChIP™ Kit.

MCF7 cells were fixed for 10 minutes with 1% formaldehyde. Chromatin was prepared using the QuikChIP™ Kit. Chromatin was sheared using a Sonifer Cell Disrupter Model W140 (Ultrasonics Inc., Plainview, NY). A volume of ~300 µl was pulsed at 100% power for 20 sec followed by a 30 sec rest on ice. The sheared and unsheared chromatin samples were reverse cross-linked and purified using phenol/chloroform extraction. The samples were run through a 1% TAE agarose gel and visualized by UV-illumination following ethidium bromide staining.

Appendix B: Purify DNA: Phenol/Chloroform

1. Add an equal volume of TE-saturated phenol to the DNA sample contained in a 1.5 mL microtube and vortex for 15-30 sec.
2. Centrifuge the sample at 12,000 rpm (7,000 x g) for 5 min at RT to separate the phases.
3. Remove about 95% of the upper aqueous layer to a clean tube, carefully avoiding proteins at the aqueous-phenol interface.
    - At this stage the aqueous phase can be extracted a second time with an equal volume of 1:1 TE-saturated phenol/chloroform, centrifuged and removed to a clean tube as above.
    - Additional extraction usually is not necessary if care is taken during the first phenol extraction.
4. Add 2 volumes of 100% EtOH and one-tenth volume of 3M NaOAc (sodium acetate), pH 5.2, to the aqueous layer and invert to mix.
5. Incubate the sample at -20°C for 1 hr to overnight.
6. Centrifuge at 12,000 rpm (7,000 x g) for 15 min at 4°C, decant the supernatant, careful not to disturb the pellet and drain inverted on a paper towel.
7. Wash the pellet with 500 µL of 70% EtOH.

8. Centrifuge the sample at 12,000 rpm (7,000 x g) for 5 min and decant the supernatant, careful not to disturb the pellet.
9. Air-dry inverted on a paper towel.
10. Resuspend dried DNA in 50 µL 1X TE buffer.

Appendix C: Trouble Shooting and FAQS

What is ChIP?

ChIP is a very versatile technique used to identify regions of the genome associated with specific proteins within the context of the cell. ChIP requires the use of a specific antibody to selectively enrich a chromatin fraction containing a specific antigen. Antibodies that recognize a protein of interest can be used to determine the relative abundance of that antigen at one or more loci in the genome.

ChIP procedure consists of the following steps:

Cells are fixed with formaldehyde to cross-link proteins to DNA. The chromatin is released from the nuclei and sheared into 200-1,000 bp fragments. The chromatin is then subjected to an immunoprecipitation, which requires the use of specific antibodies. The chromatin/antibody complex is then isolated using Protein G Agarose. After reverse cross-linking, the associated DNA is released from the complex and is ready for analysis.

Cross-linking

Cross-linking of DNA and proteins is often required to stabilize their interactions before analysis. The aim of cross-linking is to fix the antigen of interest to its chromatin binding site. Histones are generally tightly associated with DNA and may not require cross-linking. Other DNA binding proteins have a weaker affinity and need to be cross-linked to avoid dissociation from the chromatin binding site.

How do I know if cross-linking is necessary for my particular DNA-binding protein of interest?

As a general guideline, cross-linking is recommended for all non-histone DNA-binding proteins. Histones generally should not be cross-linked because they are already tightly associated with DNA. However, the need to crosslink may have to be determined empirically. Some histone proteins may be less tightly associated with DNA and require cross-linking to maintain their association with DNA. Likewise, some non-histone proteins may be tightly associated with DNA and do not require cross-linking.

How do I cross-link?

We use 1% formaldehyde, as the links it forms are reversible. UV cross-linking is irreversible.

How long should I cross-link?

Cross-linking is a time-critical procedure and should be carried out for 10 minutes. Excessive cross-linking can lead to a decrease in the amount of protein bound to the DNA and reduction in the availability of epitopes/changes in epitopes for antibody binding. In turn, this leads to reductions in the material bound/antigen available in your sample. If you are uncertain, perform a time-course experiment to optimize cross-linking conditions.

How long should I perform the cross-link reversal?

Cross-linking is reversed using 5M NaCl for 4 hours to overnight. It is important to perform this for at least 4 hours.

Chromatin fragmentation

Shearing of the chromatin into 200-1000 bp fragments is required to ensure good resolution for ChIP. If your average fragment size is greater than 1,000 bp, you could be pulling down DNA that contains your target sequence for PCR, but the protein of interest may be a significant distance from your target.

How should I determine optimal Chromatin shearing?

Please see Appendix A for complete protocol.

How do I know if my antibody is compatible with ChIP?

Antibodies are used in ChIP to capture the DNA/protein complex. Performing a successful ChIP assay requires that the antibody recognized fixed protein that is bound to the chromatin complex. Antibodies used for ChIP should be fully characterized. However, even fully characterized antibodies may not function, as the effects of cross-linking can dramatically alter protein epitopes. In general, polyclonal antibodies are preferred because the polyclonal population may recognize several different epitopes, rather than monoclonal antibodies, which only recognize a single epitope. As a rule, if an antibody works in normal immunoprecipitation, it is a good candidate for ChIP.

Novus Biologicals offers several ChIP compatible antibodies.
For a complete list please visit our web site at
www.novusbio.com.

What concentration of antibody should I use in my ChIP experiment?

The amount of each antibody will need to be empirically determined. However, if you are uncertain, 1-10 µg of antibody for every ChIP assay is a general recommended starting point.

Why do I need to wash with so many buffers?

A gradual increase in stringency in the wash buffers is necessary to prevent chromatin from interacting with the Protein G Agarose beads. This is incorporated into the wash buffers provided in the QuikChIP™ Kit. However, it may be necessary to optimize the wash buffers for each new ChIP. In general, the more stringent the buffer used, the cleaner the results with be.

Why is it difficult to pellet the Protein A/G Agarose beads?

It should not be difficult to pellet the Protein A/G Agarose beads. You should not need to centrifuge the beads at forces higher than 3,000 x g. Difficulty pelleting the beads implies that the beads have been damaged. Applying excessive force to the beads may crush or deform them. Freezing the beads will also cause damage.

Possible Controls

NEGATIVE CONTROL ANTIBODY: Either do not use a primary antibody or use a normal rabbit Ig (for rabbit antibody) or mouse IgG (for mouse antibody).

POSITIVE CONTROL ANTIBODY: To ensure that each step of the procedure is working.

NEGATIVE CONTROL PCR PRIMER: Is designed against a sequence that would not be enriched by your chromatin IP procedure.

INPUT DNA: Input DNA is DNA obtained from chromatin that has been cross-link reversed similar to your samples. It is a control for PCR effectiveness.

NOTES