

# 6 CHAPTER

## CHROMATIN IMMUNO- PRECIPITATION

### Chapter Six: Chromatin immunoprecipitation (ChIP)

Contents	Page
Chromatin immunoprecipitation overview .....	20
Chromatin immunoprecipitation formats	
Antibody format .....	21
Antibody-free format; Use of HaloTag fusion proteins .....	22
When to use chromatin immunoprecipitation .....	23
Primary reagent requirements for chromatin immunoprecipitation assays .....	23

#### Chromatin immunoprecipitation overview

Chromatin immunoprecipitation (ChIP) is an experimental method used to determine whether proteins, such as certain transcription factors, are associated with a specific genomic region in living cells or tissues. This cell-based technique is often used together with non-cell-based assays to characterize protein:DNA interactions.

The method is based on the principle that formaldehyde reacts with primary amines located on amino acids and the bases on DNA or RNA molecules, forming a covalent crosslink between the specific protein to the DNA on which they are situated.

Following crosslinking, the cells are lysed and the crude cell extracts are sonicated to shear the DNA to a smaller size. The protein:DNA complex is immunoprecipitated using an antibody against the protein of interest. The DNA protein cross-links are reversed by heating and the proteins removed by treatment with proteinase K. The DNA portion of the complex is then purified and identified by PCR using specific primers to the suspected binding region.

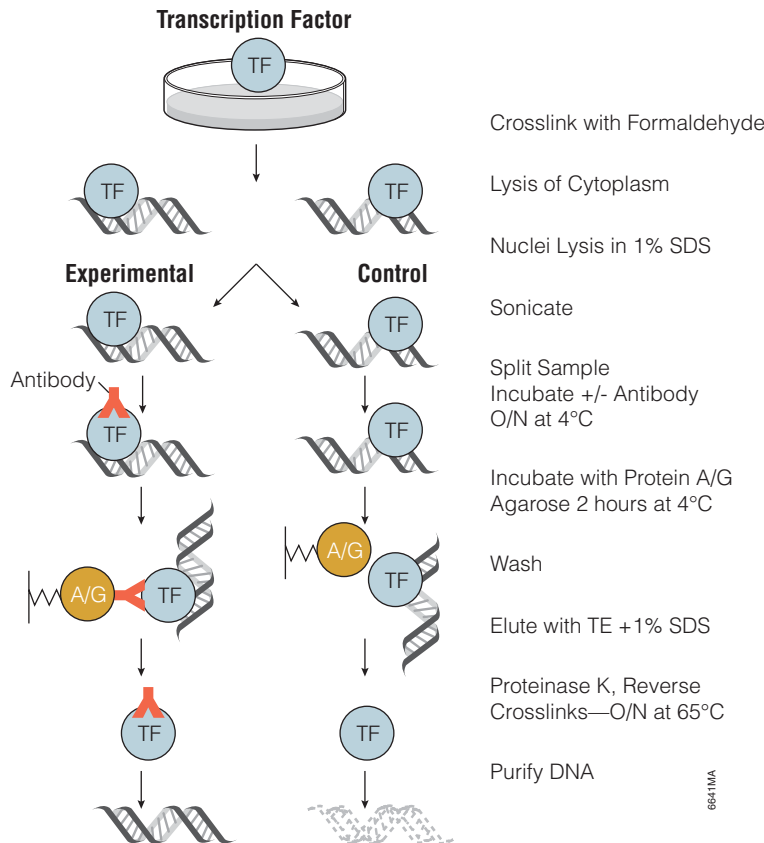


## Chromatin immunoprecipitation (ChIP) formats

### Antibody format

The classic format of the ChIP assay follows the basic procedure noted in Figure 10 and requires four days for completion. The procedure requires highly specific antibodies

to the protein of interest. If antibodies are not available the proteins can be fused to tags such as HA or c-myc, which are recognized by commercially available antibodies. The success of the procedure relies on the ability of the antibody to bind to the target protein after crosslinking (crosslinking changes epitope recognition of the antibody).



**Figure 10. Overview of chromatin immunoprecipitation using antibodies.** Mammalian cells are grown using the appropriate conditions to modulate the formation of protein:DNA interactions. To conserve the DNA:protein structure during cell lysis formaldehyde is added resulting in the formation of cross-links between the DNA and the bound protein. A whole-cell extract is prepared, and the cross-linked chromatin is sheared by sonication to reduce average DNA fragment size. Either a polyclonal or monoclonal antibody to the target protein is added. The success of the procedure relies on the use of an antibody that will specifically and tightly bind its target protein under the buffer and wash conditions used. Protein A/G agarose beads are added to capture the complex and incubated overnight. Reversal of the formaldehyde cross-linking by heating permits the recovery and quantitative analysis of the immunoprecipitated DNA.

### REFERENCES

#### Key original reference for chromatin immunoprecipitation

- Solomon, M. *et al.* (1985) *Proc. Natl. Acad. Sci.* **82**, 6470–74.

#### Antibody format

- Benson, L. *et al.* (2006) *J. Biol. Chem.* **281**, 9287–96.
- Ghosh, M. *et al.* (2006) *Mol. Cell. Biol.* **26**, 5270–83.
- White, D. *et al.* (2006) *Cancer Res.* **66**, 3463–70.
- Lei, H. *et al.* (2006) *Proc. Natl. Acad. Sci.* **103**, 10305–09.
- Shur, I. *et al.* (2006) *Stem Cells* **24**, 1288–93.
- Natesampillai, S. *et al.* (2006) *J. Biol. Chem.* **281**, 3040–47.

### TO ORDER

Phone  
1-800-356-9526

Fax  
1-800-356-1970

Online  
www.promega.com

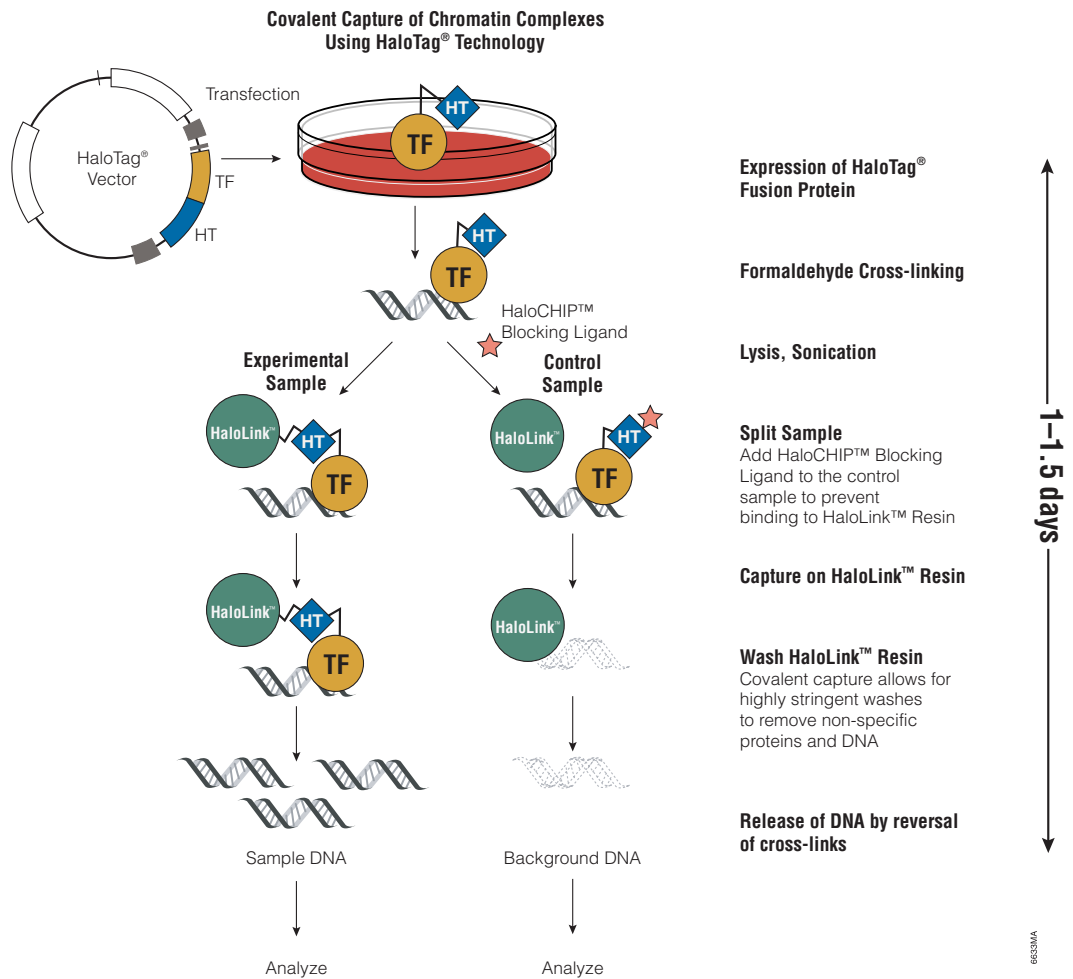


**Promega**  
www.promega.com

**Antibody-free format; use of HaloTag fusion proteins (Figure 11)**

An assay format that does not require the use of antibodies is based on the HaloTag Technology. A HaloTag vector containing the protein-coding sequence of interest is transfected into the appropriate mammalian cell line. The cells are then fixed with formaldehyde, allowing the HaloTag fusion protein to be crosslinked to the specific region

of chromatin, lysed and then sonicated. Then, instead of using an antibody to capture the DNA:protein complex, the complex is captured directly onto the HaloLink Resin. The HaloLink Resin provides a method for covalent, oriented attachment of HaloTag fusion proteins onto a solid surface. The DNA:protein crosslinks are reversed by heating, and the DNA can then be purified using commercially available columns.



**REFERENCES (CONTINUED)**

**Genome-wide chromatin immunoprecipitation assays**

1. Krieg, A. *et al.* (2006) *Mol. Cell. Biol.* **26**, 7030–45.
2. Mayanil, C. *et al.* (2006) *J. Biol. Chem.* **281**, 24544–52.
3. Kajiyama, Y. *et al.* (2006) *J. Biol. Chem.* **281**, 30122–31.

**TO ORDER**

Phone  
1-800-356-9526

Fax  
1-800-356-1970

Online  
www.promega.com



**Promega**  
www.promega.com



### When to use chromatin immunoprecipitation

Chromatin immunoprecipitation can be used to confirm the location of individual or multiple transcription factors during cell growth or upon exposure to abnormal conditions such as UV radiation. The technique can also be utilized in conjunction with microarrays to discover the location of various transcription factors on a genome-wide basis.

### Primary reagent requirements for chromatin immunoprecipitation assays

- Mammalian cell line of choice
- Cell culture media
- Polyclonal or monoclonal antibody
- Cell lysis reagent
- Formaldehyde
- Protein A/G agarose
- Proteinase K
- DNA purification reagents
- PCR reagents (including primers specific for the DNA region of interest)
- HaloTag fusion protein (specific for HaloTag procedure)
- HaloLink Resin (specific for HaloTag procedure)

# 6 CHAPTER

## CHROMATIN IMMUNO- PRECIPITATION

### TO ORDER

**Phone**  
1-800-356-9526

**Fax**  
1-800-356-1970

**Online**  
[www.promega.com](http://www.promega.com)



**Promega**  
[www.promega.com](http://www.promega.com)

