ChIP FAQs

Discover solutions to problems with your ChIP experiment with our ChIP troubleshooting tips.

1. High background in non-specific antibody controls

Non-specific binding to Protein A or G beads

Include a pre-clearing step, whereby the lysed sample is mixed with beads alone for 1 hr and removed prior to adding the antibody.

The ChIP buffers may be contaminated

Briefly spin vial before opening; inspect for undissolved material after reconstituting.

Standard degraded

Prepare fresh lysis and wash solutions

Some Protein A or G beads give high background

Find a suitable supplier that provides the cleanest results with low background in the non-specific control.

2. Low resolution with high background across large regions

DNA fragment size may be too large

DNA fragmentation should be optimized when using different cell types. Both sonication times and enzyme incubation times can vary. We would suggest a DNA fragment size of no larger than 1.5 kbp. If chromatin is being digested using enzymes, mononucleosomes (175 bp) can be obtained.

3. Low signal

The chromatin size may be too small

Do not sonicate chromatin to a fragment size of less than 500 bp. Sonication to a smaller size can displace nucleosomes as intranucleosomal DNA becomes digested. If performing N-ChIP enzymatic digestion is generally sufficient to fragment chromatin.

If performing X-ChIP, the cells may have been cross-linked for too long

Cross-link with formaldehyde for 10-15 min and wash well with PBS. Cells may need to be treated with glycine to quench the formaldehyde. Excessive cross-linking can reduce the availability of epitopes and thus reduce antibody binding.

Not enough starting material

We would suggest using 25 μg of chromatin per immunoprecipitation.

Not enough antibody included in the immunoprecipitation

We would suggest using between 3-5 μg of antibody in the first instance. This could be increased to 10 μg if no signal is observed.

Specific antibody binding is being eliminated

Do not use higher than 500 mM NaCl in the wash buffers as this may be too stringent and remove specific antibody binding.

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Cells are not effectively lysed

We would suggest using RIPA buffer to lyse cells. The composition can be found in our X-ChIP protocol.

No antibody enrichment at region of interest

The epitope is not found at the region of interest. Be sure to include a positive control antibody to confirm the procedure is working well e.g. H3K4me3/H3K9me3 antibody at active/inactive promoters.

N-ChIP is not suitable

X-ChIP may be more suitable when analyzing proteins that have either a weaker DNA affinity or are a long way from DNA. Cross-linking may be required to stop proteins dissociating from the DNA. Histones are tightly associated therefore N-ChIP can be performed when studying histones.

Monoclonal antibodies may not be suitable for X-ChIP

The epitope may have become masked during cross-linking thus preventing epitope recognition. We would suggest using polyclonal antibodies that will recognize multiple epitopes as there is an increased chance of immunoprecipitating the protein of interest.

The wrong antibody affinity beads were used

Protein A and G are bacterial proteins that bind various classes of immunoglobulins with varying affinities. Use an affinity matrix that will bind your antibody of interest. We would suggest using a mix of Protein A and Protein G that have been coupled to sepharose.

4. Problems with PCR amplification on immunoprecipitated DNA

High signal in all samples after PCR, including no template control

Real-time PCR solutions may be contaminated, we suggest preparing new solutions from stocks.

No DNA amplification in samples

Be sure to include standard/input DNA to confirm that the primers are working well.

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