QUICK TIPS FOR CHIP BEGINNERS

Guidance from Abcam Chromatin Experts on Antibody Validation and ChIP Controls

At Abcam we run quite a few ChIP assays and we interact with customers that run even more than us. Lately, we've noticed that more researchers are turning to ChIP for the first time as gene regulation studies continue to grow.

So we thought we'd share a couple of quick tips for various aspects of the ChIP method that can be helpful. For a deeper dive into the method, we have both a complete ChIP Tips Guide and a ChIP troubleshooting guide that might be useful to you as well.

IN THIS GUIDE…

ChIP Antibody Validation
Better understand how you can validate an antibody if you cannot find a ChIP-grade antibody for your target of interest.

Controls for ChIP Experiments
Learn more about useful controls to use during your ChIP assay that will streamline your experiments.

VALIDATING ANTIBODIES FOR CHIP APPLICATIONS

It's always a good idea to use an antibody that has already been validated in ChIP applications since you're asking the antibody to do something that is very different from other antibody applications. If you can't find an antibody that's already been validated in a ChIP setting, then consider the following steps:

- **Perform standard ChIP experiment**
  Try to find an antibody to your target that has been validated by immunoprecipitation, immunohistochemistry, or immunocytochemistry as they will be more likely to perform well in ChIP applications. Next, perform a standard ChIP experiment. You will need a positive and a negative control locus, i.e. a locus where you know your protein/modification of interest is present and one where you know it is absent or significantly decreased.

- **Determine the optimal antibody concentration**
  Using the correct antibody concentration can significantly improve the signal to background ratio. Titrate the amount of antibody required by performing a ChIP experiment using a range of antibody concentrations. The amount of antibody required per ChIP typically ranges from 1–10 µg of antibody for every 25 µg of chromatin.

- **Test different washing conditions**
  Some antibodies have a low affinity for the target; it is therefore worth testing different washing conditions. The stringency of the last wash can be varied from 250-500 mM salt (usually NaCl or LiCl).

- **Western Blot Verification**
  A Western blot can be used to test whether the target has been immunoprecipitated. To test if the antibody pulls down the target of interest you can perform ChIP up to the final wash after the IP and then boil the beads in loading buffer for 10 min. You can then confirm the presence of your protein in the IP by Western blot.

CONTROLS IN CHIP ASSAYS

For involved methods like ChIP, good controls are a must. When starting off with ChIP applications, you will want to make sure that you have a good plan for assessing how the experiment is proceeding at the various stages. Here are a few controls that we've used successfully:

- **Antibody Controls**
  As a positive antibody control for ChIP, Histone H3 (tri methyl K4) is a popular positive control to use when chipping active genes. As a negative control, use an antibody that recognizes a non-chromatin epitope such as an anti-GFP antibody.

- **Negative controls**
  As a negative control, use `beads only` or beads with an isotype matched control immunoglobulin (Ig), this will give you the background of the assay. For example, ab26721, a RNA polymerase II CTD repeat YSPTSPS-ChIP grade antibody, is a mouse monoclonal IgG1. A suitable isotype control would be ab18443, mouse IgG1, Kappa monoclonal isotype control.

- **Positive and negative control loci**
  Primers for a locus where you know your protein or modification of interest is present (positive control locus) and one where it is absent or significantly decreased (negative control locus) should both be tested in RT-PCR. This will tell you whether the observed enrichment is specific. It is important to include these controls as some antibodies result in non-specific enrichment.

- **Non-template control**
  A non-template control should always be included in the PCR reaction. This will help you spot any contamination.

- **Positive control antibody**
  Antibodies specific for Histone H3 (tri methyl K4) (ab8580) and H3 (acetyl K9) (ab4441), both enriched on actively transcribed regions, are good controls to ensure that each step of your experiment is working.

ABCAM CHIP EXPERTS

Meet some of our ChIP specialists. These scientists have years of experience in the lab and helping customers.

Rachel Imoberdorf, PhD
Sr Core Product Team Leader

ChIP Fact: I have ChIP'ed more than 4,000 antibodies, have analyzed over 10,000 ChIP graphs and my favorite ChIP antibody still is ab8580."

I lead the team that is responsible for the quality control of our antibodies. My first project at Abcam (7 years ago) was to set up ChIP in our laboratory so that we can test our antibodies in this application. The result of this and many other optimization projects is our high quality, ChIP batch tested, range of histone antibodies. Even though I don't do ChIP anymore myself I still love this technique.

Top Tip: Histone antibodies usually work well in ChIP. Ensure the specificity of these antibodies is tested in peptide array, peptide blocking western blot, or ELISA.

Karen Halls, PhD
Scientific Support Manager

ChIP Fact: I've been ChIP'ing for the past 10 years!

I work within the Abcam Scientific Support team. We're always available to answer questions and give advice. If you are not sure which product is right for you, or would like a bit more information just give us a call. We are continually adding Images, Abreviews and References to the datasheet, everything we know about the product will be online. Our products are fully guaranteed to work as described on our datasheets so don't hesitate to contact us as we are here to help.

Top tip: When selecting an antibody for ChIP, I check whether an antibody is affinity purified. This should minimize any cross-reactivity with other proteins and ensure the signal is representative of the target protein.

Sonja Flott, PhD
Epigenetics Business Development

ChIP Fact: I've attended over a dozen conferences in the last 3 years to get customer feedback on what antibodies they need.

I look after our Epigenetics & Nuclear Signaling products from a business development point of view - coming up with new product ideas, working on improving the characterization of our products and ensuring quality control. I attend conferences to network with researchers and try to stay on top of literature to find hot topics & to get help with characterization. We are always working on increasing our range of batch tested ChIP antibodies. I look through many publications to find the most appropriate ChIPing conditions, and I always appreciate feedback from scientists. So if you would like us to test our antibodies to your favourite protein in ChIP, let me know.

Top tip: My favorite indicator that an antibody will work in ChIP, RIP or CLIP is that it works in IP. So, my first choice antibodies for ChIP are always antibodies that work in IP.

abcam®
discover more