CONSIDERATIONS FOR CHOOSING EFFECTIVE ChIP ANTIBODIES

With the expertise of Upstate® and Chemicon®, EMD Millipore has developed a comprehensive line of kits, assays, and antibodies for ChIP, so we understand the importance of choosing the right antibody for your ChIP experiment. In this guide, our research experts will share critical considerations for selecting an effective ChIP antibody.

MONOCLONAL VS. POLYCLONAL ANTIBODIES

Either monoclonal or polyclonal antibodies will work for ChIP.

Because monoclonals recognize a single epitope on a target protein, they often provide a high level of specificity, low non-specific binding, and low background signals. In addition, monoclonals generally perform more consistently from batch to batch due to low variability in their clonal nature. However, if the epitope recognized by the monoclonal is not accessible due to the presence of other chromatin associated proteins or is masked by steps in the ChIP protocol (e.g. cross-linking) then monoclonals will not effectively bind to the protein. Fortunately, masking is not common, so monoclonals are suitable for ChIP and can yield excellent results.

In contrast to monoclonals, polyclonal antibodies typically recognize multiple epitopes on a target protein, and can be more effective at detecting a target even if a few epitopes are masked by cross-linking. However, because polyclonals recognize multiple epitopes, this can increase the probability that nonspecific binding will occur. In addition, the specificity of a polyclonal antibody can drift, unless the serum from which the antibody is derived is pooled prior to preparation or purification.

To mitigate these challenges, EMD Millipore immunizes multiple animals followed by screening and pooling of sera demonstrating appropriate affinity and specificity. To ensure consistency, performance of the final antibody can be compared to previous batches.

CONSIDER PERFORMANCE DATA FROM OTHER IMMUNOASSAYS

Not all antibodies have data demonstrating performance in ChIP, but that does not always indicate that they will not work in ChIP. To save time screening multiple candidate antibodies, consider data from other immunoassays as an indicator of the likelihood that an antibody will work well in ChIP.

Western blot and immunoprecipitation assays will tell you if the antibody recognizes the correct target; and immunofluorescence analyses will help you determine if the antibody recognizes the target in its native state, which is important for ChIP. An antibody validated in multiple applications such as immunoprecipitation, immunofluorescence or immunohistochemistry is more likely to produce positive ChIP results than an antibody validated for only Western blot. Of course performance in these applications is not a guarantee of success in ChIP, as successful ChIP antibodies must recognize accessible epitopes that are not affected by cross-linking methods often used in ChIP.

If ChIP data is available for the antibody consider the following questions. Is the data generated with the same lot of antibody you will be using or was it published using a different lot? Have others used this antibody for ChIP? Were both known positive and known negative locations used to verify enrichment?

Because antibody performance can vary the ideal antibody to use for a ChIP experiment is well characterized in terms of specificity and performance in multiple applications, is validated in ChIP and is lot tested in the ChIP application. EMD Millipore's ChIPAb+ antibodies meet these criteria and include a negative IgG control and positive control primer set for verification of enrichment.

CHOOSE A ChIP-VALIDATED ANTIBODY FROM OUR ChIPAb+™ COLLECTION:

- Every batch proven and quality controlled for ChIP
- Validated in multiple immunoassays
- qPCR primers and negative control IgG included to validate your results

Discover EMD Millipore's complete line of ChIPAb+™ antibodies at **www.millipore.com/epigenetics**

VERIFY ANTIBODY SPECIFICITY

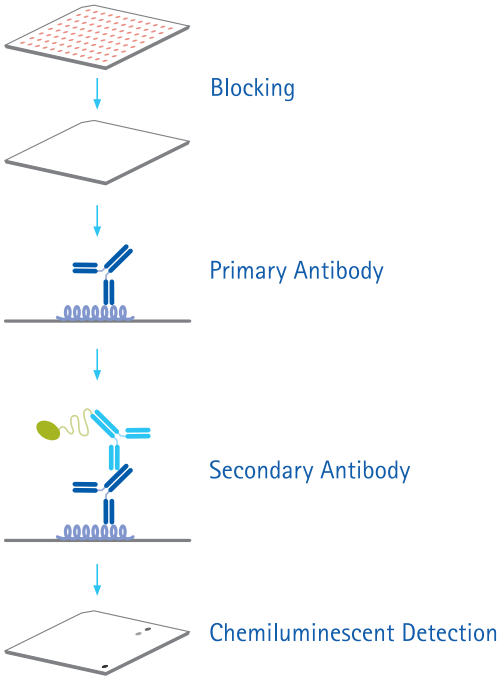
Before finalizing your antibody selection and performing your experiment, it's important to evaluate the specificity of your antibody. This is particularly true with histone antibodies. Unlike EMD Millipore, not all commercial vendors rigorously test their histone antibodies for specificity. In addition, reviewers of journal submissions are increasingly requesting demonstrations of histone antibody specificity as a condition of publication, so it is important to perform independent screening assays to detect potential cross reactivity. Specificity of an antibody can be evaluated in following assays:

- Peptide Dot Blots, such as EMD Millipore's AbSurance™ Arrays (Cat. No. 16-665, 16-667, and 16-668)
- Peptide Inhibition Assays: typically for antibodies to specific modification states
- Peptide Microarrays
- Peptide interaction assays using xMAP® beads or other plate-based assays

To learn more about the histone antibody specificity screening or AbSurance Histone Peptide Arrays please refer to these two articles on the EpiGenie website.

- AbSurance™ Histone Peptide Arrays: Insurance for Your Antibodies
- Getting Down to Histone Antibody Specifics

Simple Specificity Screening with the AbSurance™ Histone Peptide Arrays



1. Select appropriate AbSurance™ Array.
2. Incubate the array in blocking solution.
3. Incubate with your histone antibody.
4. Detect interactions using secondary antibody and a chemiluminescent system.
5. Inspect with alignment tools – no special array imaging equipment or software required.

KEY POINTS FROM THIS GUIDE

1. Both monoclonals and polyclonals can work in ChIP
2. Ideal antibodies are ChIP-validated.
3. Antibodies validated in multiple immunoassays such as IP or IHC more frequently work in ChIP than antibodies validated in only Western blots.
4. Perform independent specificity screening.

For more information on selecting the right antibody for ChIP, or other ChIP-related questions, please contact EMD Millipore Technical Support or your local representative.

www.millipore.com/epigenetics

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