Application Forum

Utilization of Peptide Macroarrays for Evaluating Specificity of Antibodies to Modified and Unmodified Core Histones

Introduction

Gene expression, DNA repair, and cell-cycle progression are tightly regulated by changes in chromatin structure and stability (1,2). These changes in chromatin are partially dependent on reversible post-translational modifications (PTMs) occurring on the N-terminal tails of core histones, H2A, H2B, H3, and H4. PTMs alter the chemical nature of histones and produce docking sites for a vast array of chromatin-modifying enzymes, transcriptional complexes, and signaling proteins. More than 100 histone modifications have been identified, among which methylation, acetylation, and phosphorylation have emerged as key epigenetic signaling mechanisms.

It is increasingly clear that histone modifications may cooperate or antagonize each other, and even differences of one chemical group (di- vs. trimethylation, for example) may produce a different biological outcome. Thus, understanding the effects of a single type of PTM, as well as the spatiotemporal effects of several different PTMs, often termed the “histone code,” is an important aspect of epigenetics research that allows additional and sometimes subtle characterization of cellular signaling states.

Currently, analyses of histone PTMs relies heavily on chromatin immunoprecipitation (ChIP). In ChIP, an antibody against a specific histone PTM or unmodified histone is used to pull-down associated DNA sequences, which are further analyzed by quantitative PCR, microarrays (ChIP-chip), or next-generation sequencing (ChIP-seq). ChIP can be a powerful strategy to gain both locus-specific and genome-wide information, but only when using highly specific and well-characterized histone antibodies.

A large percentage of available ChIP antibodies are offered by commercial suppliers, and are often viewed as more fail-safe options. However, unlike EMD Millipore, not all suppliers rigorously or consistently screen their histone antibodies for cross reactivity. In addition, independent studies have demonstrated that a significant percentage of commercial ChIP antibodies can also bind to non-specific targets (4,5), such as unmodified histones, similar adducts, and proximal signaling proteins.

Commercial polyclonal antibodies may be particularly problematic for ChIP, as specificity may drift during immunization and differ significantly from batch to batch (depending

on material-handling practices of the supplier). Therefore, independent screening of the cross-reactivity profile of histone antibodies has become an essential step in ChIP-based studies.

Here we describe two simple screening arrays for evaluating the specificity of antibodies directed against unmodified histones and key histone modifications (acetyl; phospho; mono-, di-, and trimethyl), featured in a recent high-profile publication (4). In contrast to peptide arrays that are currently available, the AbSurance™ Antibody Specificity Arrays are user-friendly, cost-effective, and are designed for rapid specificity screening without specialized detection equipment or software.

The AbSurance™ arrays comprise 89 synthetic modified and unmodified peptides based on human histone protein

Figure1. Intuitive, convenient, Western blot-like protocol.

Figure 2. AbSurance™ arrays detect specific and nonspecific interactions. Detection of specific interactions using EMD Millipore's antibody to trimethyl histone H3 lysine 4 (Catalogue No. 05-745R). AbSurance™ array shows expected specificity for H3K4me3 peptide (9A and 9B, green box) without cross-reaction to other peptides including unmodified H3, H3K4 (6A and 6B), H3K4me (7A and 7B), and H3K4me2 (8A and 8B). Primary antibody used at 1:2,000 dilution. Positive control rabbit IgG shown at position 12H (blue box).

Figure 3. AbSurance™ arrays detect specific and nonspecific interactions. Detection of nonspecific interactions using an antibody to acetyl histone H3 lysine 56 obtained from Supplier E. AbSurance™ array data demonstrate weak recognition of the target H3K56ac peptide (6G and 6H, green box), but strong crossreactivity with H3K9ac (1C and 1D, red box). Primary antibody used at 1:2,000 dilution. Positive control rabbit IgG shown at position 12H (blue box).

sequences. Using a proprietary process, we spotted the peptides onto a PVDF membrane in 10 and 100 ng quantities, to enable detection of both weak and strong cross-reactivity. Each lot of the Absurance™ arrays undergoes quality control Western blot testing to confirm consistency of spotting as well as the identity of spotted peptides. The arrays also provide internal positive control primary antibodies from rat, mouse, rabbit, and sheep, which are strategically positioned at the bottom-right corner to also serve as an additional landmark for spot identification. In addition, the PVDF membrane platform enables a simple assay protocol with standard Western blot reagents and chemiluminescence detection systems, to sensitively detect antibody cross-reactivity.

Method

AbSurance™ Histone H3 Antibody Specificity arrays were used as described in the product manual. Briefly, Immobilon P (PVDF) membranes were rehydrated by immersion in 20 mL of methanol, and blocked from non-specific protein binding with 20 mL of blocking solution. One membrane was probed with a 1:1, 2000 dilution of EMD Millipore's anti-trimethyl histone H3 (Lys4) (Cat. #05-745R). The second membrane was incubated with a similar concentration of anti-acetyl histone H3 (Lys56) obtained from Competitor E. Reactive peptides were visualized with Immobilon® Western Chemiluminescent HRP substrate followed by exposure to a CCD imager and identified using the alignment grid and peptide maps, provided with the AbSurance™ arrays.

Results

Reliable detection of specific and on-specific interactions

The AbSurance™ H3 array was used to evaluate the specificity of two histone antibodies, and confirmed specific

(Figure 2) and non-specific (Figure 3) interactions, similar to results obtained from other labor-intensive screening protocols, such as peptide blocking or ELISA.

Conclusion

The AbSurance™ Antibody Specificity Arrays are simple and effective peptide membranes that can be used to characterize antibodies to key post-translational modifications of histones H2A, H2B, H3, and H4. In sensitive, antibody-dependent analyses, such as ChIP, the AbSurance™ arrays can help to confirm the validity of biological data.

References

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For more information on AbSurance™, or any of EMD Millipore's epigenetics products, please visit the website at www.millipore.com/epigenetics.

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