Hitting the mark with histone antibodies

Specificity analysis of antibodies that recognize histone posttranslational modifications.

The nucleosome is the fundamental repeating unit of the eukaryotic chromosome. It functions to package DNA into units of ~150 base pairs wrapped around two copies each of histones H2A, H2B, H3, and H4, while also significantly contributing to the regulation of gene expression. Histones have a remarkable assortment of posttranslational modifications (PTMs)—including methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, and ribosylation [1]. Due to the large number of modified histone residues, and the additional complexity resulting from the different methylation states of lysine or arginine residues, studying the epigenome requires a set of highly specific and validated tools [1].

Antibodies specific for histone PTMs are essential reagents in a variety of experimental techniques, including chromatin immunoprecipitation (ChIP), western blotting, immunofluorescence, and immunohistochemistry. ChIP is extensively used to assess protein–DNA interactions and to analyze the occupancy of chromatin modifications on a genome-wide scale. Because certain histone modifications may display similar DNA-binding patterns, the accuracy of a ChIP experiment depends upon the specificity of the antibody and its ability to distinguish between subtly different PTMs, such as dimethylation versus trimethylation. Recent studies aimed at testing the quality of commercially available histone PTM antibodies have raised concerns regarding their specificity, which is of paramount importance when analyzing the association of histone modifications and disease [2-5]. Thus, rigorous specificity analysis and functional validation of histone PTM antibodies are needed.

Here we describe our analysis of Invitrogen™ histone PTM antibodies. We are conducting extensive specificity testing of our antibodies using peptide arrays and functional ChIP validation assays, and comparing their performance to that of other widely cited, commercially available histone PTM antibodies. Table 1 lists a set of recently tested Invitrogen histone PTM antibodies that performed as well as or better than corresponding histone PTM antibodies from other manufacturers in histone peptide arrays; a detailed explanation of how this comparison was performed is discussed in Figure 1.

Table 1. Invitrogen histone PTM antibodies validated on peptide arrays.

Antibody target	Abbreviated target name	Host and class	Cat. No.*
Methylation antibodies			
Methyl-Histone H3 (Lys4)	H3K4me1	Rabbit monoclonal	701763
		Rabbit oligoclonal	710795
Methyl-Histone H3 (Lys9)	H3K9me1	Rabbit oligoclonal	710814
Methyl-Histone H3 (Lys27)	H3K27me1	Rabbit polyclonal	491012
Di-Methyl-Histone H3 (Lys4)	H3K4me2	Rabbit monoclonal	701764
		Rabbit oligoclonal	710796
Di-Methyl-Histone H3 (Lys9)	H3K9me2	Rabbit polyclonal	491007
Di-Methyl-Histone H3 (Lys36)	H3K36me2	Rabbit monoclonal	701767
Tri-Methyl-Histone H3 (Lys9)	H3K9me3	Rabbit polyclonal	491008
Tri-Methyl-Histone H3 (Lys27)	H3K27me3	Rabbit monoclonal	MA511198
Acetylation antibodies			
Acetyl-Histone H3 (Lys9)	H3K9ac	Rabbit monoclonal	701269
		Rabbit oligoclonal	710293
Acetyl-Histone H3 (Lys14)	H3K14ac	Rabbit polyclonal	720094
Acetyl-Histone H3 (Lys18)	H3K18ac	Rabbit polyclonal	720095
Acetyl-Histone H4 (Lys8)	H4K8ac	Rabbit polyclonal	720105
		Rabbit oligoclonal	710828
Phosphorylation antibodies			
Phospho-Histone H3 (Ser10)	H3pS10	Rabbit monoclonal	701258
Phospho-Histone H4 (Ser1)	H4pS1	Rabbit polyclonal	720100

*All antibodies listed here perform as well as or better than corresponding antibodies from other suppliers.

Methods for comparing histone PTM antibodies

To compare the specificity of antibodies for a particular histone PTM, we tested antibodies from various manufacturers using histone peptide arrays, which contain 384 peptides from the N-terminal tails of histones featuring 59 posttranslational modifications. This peptide array assay was followed by an analysis of the functional performance of the histone PTM antibody in ChIP. Commercially available antibodies were chosen based on citations and their applicability in peptide arrays, peptide dot blots, ChIP-qPCR, and ChIP-Seq, as well as in additional applications such as western blotting and immunocytochemistry.

Specificity analysis using peptide arrays

Figure 1A shows representative peptide arrays that have been incubated with either the Invitrogen™ Di-Methyl-Histone H3 (Lys4) Antibody (anti-H3K4me2 rabbit oligoclonal antibody) or a commercially available antibody from another supplier purported to have the same specificity.

The Invitrogen anti-H3K4me2 antibody only binds to peptides that contain the H3K4me2 modification, whereas the other supplier's antibody binds to peptides that contain the specified modification as well as peptides containing other modifications (Figure 1A). The data can also be represented as a graph of the "specificity factor" for each modification, which is the ratio of the average intensity of all spots containing a particular PTM to the average intensity of all spots lacking that PTM on the peptide array (Figure 1B). "Specific" antibodies are defined as those showing greater than a two-fold difference in the specificity factors for binding at the target site versus at the best nontarget site. Invitrogen antibodies that performed as well as or better than competitors showed equal or higher fold differences, respectively, between the specificity factors for binding at the target and at the best nontarget site (listed in Table 1).

Functional analysis in ChIP assays

Chromatin pull-down in a ChIP assay is evidence of the presence or absence of a specific histone PTM at a particular genomic locus. Use of a specific histone PTM antibody helps ensures that chromatin is not pulled down by nonspecific interactions. As this assay requires recognition of the modification in the context of nucleosomes, functional validation of a histone PTM antibody for use in ChIP is required to establish both that target epitopes are accessible and that the antibody is binding to expected loci.

Figure 1C shows the performance of the two anti-H3K4me2 antibodies in a ChIP assay. The Invitrogen anti-H3K4me2 antibody shows the expected enrichment of H3K4me2 on the active but not the silent loci, whereas the other supplier's anti-H3K4me2 antibody shows much lower fold enrichment of the active loci as compared with the silent loci. These data clearly emphasize that antibodies for ChIP should be chosen not only for their ability to pull down chromatin but also for their ability to show enrichment of target histone PTMs at the expected genomic regions.

Find the right antibody for your experiments

When choosing a histone PTM antibody, two major considerations are the stringent verification of antibody specificity and the functional validation in ChIP. To address reports of the lack of reproducibility in commercially available histone PTM antibodies [2-5], histone PTM antibodies in the Invitrogen portfolio are undergoing extensive specificity verification and functional ChIP validation.* This comprehensive study underscores the breadth of histone PTM antibodies offered by Thermo Fisher Scientific and should serve as a useful guide when choosing the right antibody for a particular experiment. To learn more about individual histone PTM antibodies or search our entire portfolio of over 80,000 antibodies, visit **thermofisher.com/antibodies**. ■

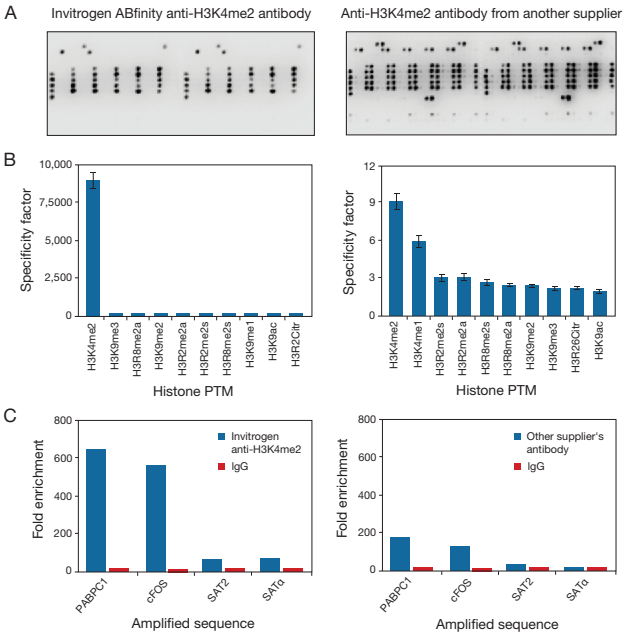

Figure 1. Specificity analysis and functional validation of two anti-H3K4me2 antibodies. (A) The specificity of the Invitrogen™ ABfinity™ Di-Methyl-Histone H3 (Lys4) Antibody (anti-H3K4me2 rabbit oligoclonal antibody, Cat. No. 710796) and a corresponding antibody (right) from another supplier was determined using MODified™ Histone Peptide Arrays (Active Motif Inc.) and manufacturers' protocols. **(B)** The results of the peptide array analysis are displayed as graphs of specificity factor (ratio of the average intensity of all spots containing H3K4me2 to those lacking H3K4me2) vs. modification. **(C)** ChIP analysis was performed on sheared chromatin from 2 x 10^6 HeLa cells using the Applied Biosystems™ MAGnify™ Chromatin Immunoprecipitation System (Cat. No. 492024); nonspecific rabbit IgG was used as a negative control. The purified DNA was analyzed using the Applied Biosystems™ 7500 Fast Real-Time PCR System (Cat. No. 4351106) with optimized PCR primer pairs for the promoters of the active PABPC1 and cFOS genes (positive controls) and for the regions of the inactive SAT2 and SATα satellite repeats (negative controls). Data are presented as fold enrichment of the antibody signal vs. signal from the negative control IgG.

*The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. The product(s) was not validated for clinical or diagnostic use.

References

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