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**Histone modification antibody database**

Antibodies that recognize modified histones are essential tools in epigenetic research. Histone modification antibodies in particular require careful validation as those often share the same epitope amino acid sequence, but only slightly differ between the possible post-translational modifications (PTM), e.g. acetylation (ac), mono-methylation (me1), di-methylation (me2), tri-methylation (me3), phosphorylation (ph), ubiquitination (ub) and others, as well as combinations thereof.

Although manufacturers extensively characterize antibodies for many different applications (e.g. immunoblot, immunofluorescence, chromatin-immunoprecipitation, etc.) before market launch, still the problem is a lack of an uniform standard operating procedure for full validation in order to gain knowledge about antibodies’ on- and off-target binding essential for interpretation of experimental data. Effort to characterize histone PTM antibodies have been published previously ([Bock et al., 2011](#_ENREF_1); [Egelhofer et al., 2011](#_ENREF_2); [Heubach et al., 2013](#_ENREF_3)), however, different methods have been applied that do not allow a thorough comparison of antibody validation results.

Our contribution to the epigenetics research community is a comprehensive characterization of specificities of 125 commercially available histone antibodies raised against x different modifications by a quantitative peptide-based dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) provided as an open web resource. Aim of this website is to share validation data as a guide for choosing suitable antibodies for different applications. We describe on-target binding and cross-selectivities of antibodies and categorized histone PTM antibodies based on their specificity and sensitivity. Moreover, we provide an unique filtering function for search queries.

We profiled the binding of mainly monoclonal antibodies against a panel of x synthetic histone modification peptides, as monoclonal antibodies have the advantage of consistency among different lots, while inherent variability between different lots of the same polyclonal antibody due to the immunological state of the animal has been reported. To summarize, one third of tested antibodies are specific for their respective histone PTM, whereas most antibodies failed our criteria. We advise to carefully characterize antibodies before use.

Figure 1: Assessment of histone PTM antibody selectivities.

A| Antibody validation by peptide-based quantitative dissociation enhanced lanthanide fluorescence immunoassay. To identify a set of highly specific histone modification antibodies commercially available antibodies were tested against a panel of x different synthetic histone post-translational modification (PTM) peptides in a quantitative dissociation enhanced lanthanide fluorescence immunoassay (DELFIA) assay format. Briefly, biotinylated (B) peptides representing different parts of proteins were immobilized to 384-well streptavidin (SA) coated plates and the binding of antibodies to peptide epitopes detected. Therefore, a two-layer detection was performed: peptides were incubated with the antibody to be tested and an anti-species Europium (EU) conjugated secondary antibody. Following washing steps, the time-resolved fluorescence of a Europium chelate is measured on a plate reader.

B| Heatmap of antibody selectivities of all tested antibodies. Heatmap displaying all tested combinations of antibodies (x-axis) versus peptides (y-axis). Fluorescence intensities from each DELFIA assay were normalized to the maximum median value of all quadruplicates. In order to establish a median fluorescence intensity threshold to distinguish real signals from noise, we applied k-mean clustering with 2 different clusters ("signal", "noise") to the median values of each independent assay. Thresholds were fixed as the maximum value of the "noise" clusters, and used to determine true positives TP (on-target binding), false negatives FN (on-target non-binding), false positives FP (off-target binding) and true negatives TN (off-target non-binding). Normalized signals are shown (red 1, blue 0).

C| Antibody classifications according to sensitivity and selectivity. Pie chart representing 125 tested commercially available histone PTM antibodies classified into 9 groups according to their sensitivity and selectivity for their cognitive epitopes. Antibodies were classified into 9 categories based on two different criteria: positive predictive value PPV=TP/(TP+FP), used as a proxy for specificity, and sensitivity S=TP/(TP+FN). PPV and S values were considered to be "high" if > 0.75 and "low" if < 0.5; otherwise "moderate". PPV values were finally adjusted to account for the difference in magnitude between the median of all true and the median of all false positive signals. Highly specific antibodies were assigned to categories 1-3 (from more to less sensitive), antibodies of average specificity to categories 4-6, and unspecific antibodies to categories 7-9. For not classified antibodies the respective epitope peptide was not tested.

D| Specific histone antibodies identified. Heatmap displaying only category 1 – 3 antibodies. Only peptides with single PTMs are displayed. Normalized signals are shown (red 1, blue 0).

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**Search**

Data on specificities of 125 commercial histone modification antibodies for x modified peptides are assembled in an open web source database with graphical display and filtering functions. Specificity and sensitivity: absence of primary epitope (false positives, FP), loss or reduction of binding although PTM is present (false negative, FN). Also less specific antibodies might be suitable for certain application, e.g. in biochemical assays off-targets may be neglected, as those are not in the test tube. The users should take the validation data as a guide.

Graphical representation of selectivity plots: x-axis different peptides tested, y-axis signal intensity measured, on-targets to be detected by respective antibody are highlighted in blue. The antibody table gives information on the histone PTM antibody, CatNr, LotNr, company, and category. The peptide table give information on the peptide sequence. Heatmap as graphical output. Download graph as .pdf file.

**Search filters**

Search queries for histone, PTM, CatNr.

**Search by modification**

**Search by antibody**

**Compare**

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**Materials and Methods**

**Dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA)**

Biotinylated peptides (New England Peptide, Inc.) at > 90% purity were dissolved in UltraPureTM DNase/RNase-Free Distilled Water (Gibco, CatNr 10977035) to obtain 10 mM stocks. 20 µl of a 1 µM peptide solution were immobilized onto DELFIA® Streptavidin-coated white 384-well plates (Perkin Elmer, CatNr CC11-H10) in quadruplicates. After 1 hour incubation at room temperature plates were washed three times with 100 μl wash buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween20), and were incubated with 50 µl antibodies at indicated dilutions in 1X FI buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween40, 25 mM DTPA, 0.2% BSA, 0.05% bovine γ-globulins) and 200 ng/ml Eu-N1-conjugated anti-species antibodies (DELFIA® Eu-N1 Rabbit anti-Mouse IgG antibody, Perkin Elmer, CatNr AD0207; DELFIA® Eu-N1 Goat anti-Rabbit IgG antibody, Perkin Elmer, CatNr AD0106) for 1 hour at room temperature. After removal of unbound antibodies by washing the plates three times with 100 μl wash buffer, plates were incubated with 50 μl DELFIA® Enhancement Solution (PerkinElmer, CatNr 4001-0010) for 45 minutes at room temperature. Europium emission (Emission 615/8.5 nm; Excitation 340/60nm) was measured using an EnVision 2104 Multilabel Plate Reader (Perkin Elmer).

**Data normalization and statistics**

Fluorescence intensities from each DELFIA assay were normalized to the maximum median value of all quadruplicates. In order to establish a MFI threshold to distinguish real signals from noise, we applied k-mean clustering with 2 different clusters ("signal", "noise") to the median values of each independent assay. Thresholds were fixed as the maximum value of the "noise" clusters, and used to determine true positives TP (on-target binding), false negatives FN (on-target non-binding), false positives FP (off-target binding) and true negatives TN (off-target non-binding). Antibodies were classified into 9 categories based on two different criteria: positive predictive value PPV=TP/(TP+FP), used as a proxy for specificity, and sensitivity S=TP/(TP+FN). PPV and S values were considered to be "high" if > 0.75 and "low" if < 0.5; otherwise "moderate". PPV values were finally adjusted to account for the difference in magnitude between the median of all true and the median of all false positive signals. Highly specific antibodies were assigned to categories 1-3 (from more to less sensitive), antibodies of average specificity to categories 4-6, and unspecific antibodies to categories 7-9. Data are visualized using R script ggplot, with median values shown with standard error bars.

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**About us**

Author distributions

Erika Schirghuber performed experimental work. Database and website design: Adrián César Razquin established the web page with input from Peter Majek, Alexey Stukalov, Jacques Colinge and Stefan Kubicek. The website is maintained by Michael Pilz from CeMM’s IT department.

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