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This document presents validation data for two histone antibodies – H3.3 and H2A.Z.

The validations rely on western blots. We believe that these blots satisfy primary and secondary validation criteria. The western blots show that each antibody binds an epitope of the expected size (primary validation) and does not bind to a recombinant source of the corresponding canonical histone, and is thus specific to the non-canonical histone variant (secondary validation). Because these are antibodies to histone variants, rather than post - translational modifications of histones, we do not use our array of modified peptides.

Details for each antibody are provided on the following pages.

H3.3 (Millipore 09-838 lot # 2273984)

https://www.encodeproject.org/antibodies/ENCAB494QXU/

In Panel A, we demonstrate that the recombinant proteins purchased from NEB actually exist by staining with a general histone H3 / H3.3 antibody (positive control). This antibody detects all H3 variants.

In Panel B, we demonstrate the specificity of the H3.3 antibody, by showing that it ONLY RECOGNIZES histone H3.3 and not Histone H3.1 and H3.2. This is our SECONDARY VALIDATION.

In Panel C, we demonstrate that the antibody we seek to validate recognizes proteins of the appropriate apparent molecular weight (gel migration) in a western blot using nuclear extracts from K562 cells and HEK293 cells. This is our PRIMARY VALIDATION. The graphical image from our validation is reproduced here (Figure 1). In addition (in Figure 2) we share a validation image to a different lot of H3.3 antibody that is not yet accessioned at the DCC, but which is currently in transit to the DCC, and follows the exact same principle.



Figure 1 Primary and Secondary Validation of Millipore antibody to H3.3



Figure 2 Primary and Secondary Validation of Millipore antibody to H3.3, part 09-838, lot# 2202506

H2A.Z (Millipore 07-594 lot # DAM1540736)

https://www.encodeproject.org/antibodies/ENCAB000ASY/

In Panel A, we demonstrate that the recombinant protein (H2A) purchased from NEB actually exists, by staining with a general histone H2A antibody (positive control). This recombinant protein co-migrates on a gel with the H2A found in a HEK nuclear lysate (positive control).

In Panel B, lane 1, we demonstrate the specificity of this H2A.Z Antibody, by showing that it FAILS TO RECOGNIZE the recombinant histone H2A. This is our SECONDARY VALIDATION.

In Panel B, lanes 2 and 3, we demonstrate that this H2A.Z antibody recognizes a protein of the appropriate molecular weight (gel migration) in a western blot using nuclear extracts from K562 cells and HEK293 cells. This is our PRIMARY VALIDATION.



Figure 3 Primary and Secondary Validation of Millipore Antibody to H2A.Z

H2A.Z (Millipore 07-594 lot # DAM176448)

https://www.encodeproject.org/antibodies/ENCAB000BKS/

In Panel A, we demonstrate that the recombinant protein (H2A) purchased from NEB actually exists, by staining with a general histone H2A antibody (positive control). This recombinant protein co-migrates on a gel with the H2A found in a HEK nuclear lysate (positive control).

In Panel B, lane 1, we demonstrate the specificity of this H2A.Z Antibody, by showing that it FAILS TO RECOGNIZE the recombinant histone H2A. This is our SECONDARY VALIDATION.

In Panel B, lane 2, we demonstrate that this H2A.Z antibody recognizes a protein of the appropriate molecular weight (gel migration) in a western blot using a nuclear extract from HEK293 cells. This is our PRIMARY VALIDATION.



Figure 4 Primary and Secondary Validation of Millipore Antibody to H2A.Z