

ENCODE DCC Antibody Validation Document

Date of Submission

Name:

Email:

Lab

Antibody Name:

Target:

Company/
Source:

Catalog Number, database ID, laboratory

Lot Number

Antibody Description:

Target Description:

Species Target

Species Host

Validation Method #1

Validation Method #2

Purification Method

Polyclonal/
Monoclonal

Vendor URL:

Reference (PI/
Publication
Information)

Please complete the following for antibodies to histone modifications:
if your specifications are not listed in the drop-down box,
please write-in the appropriate information

Histone Name

AA modified

AA Position

Modification

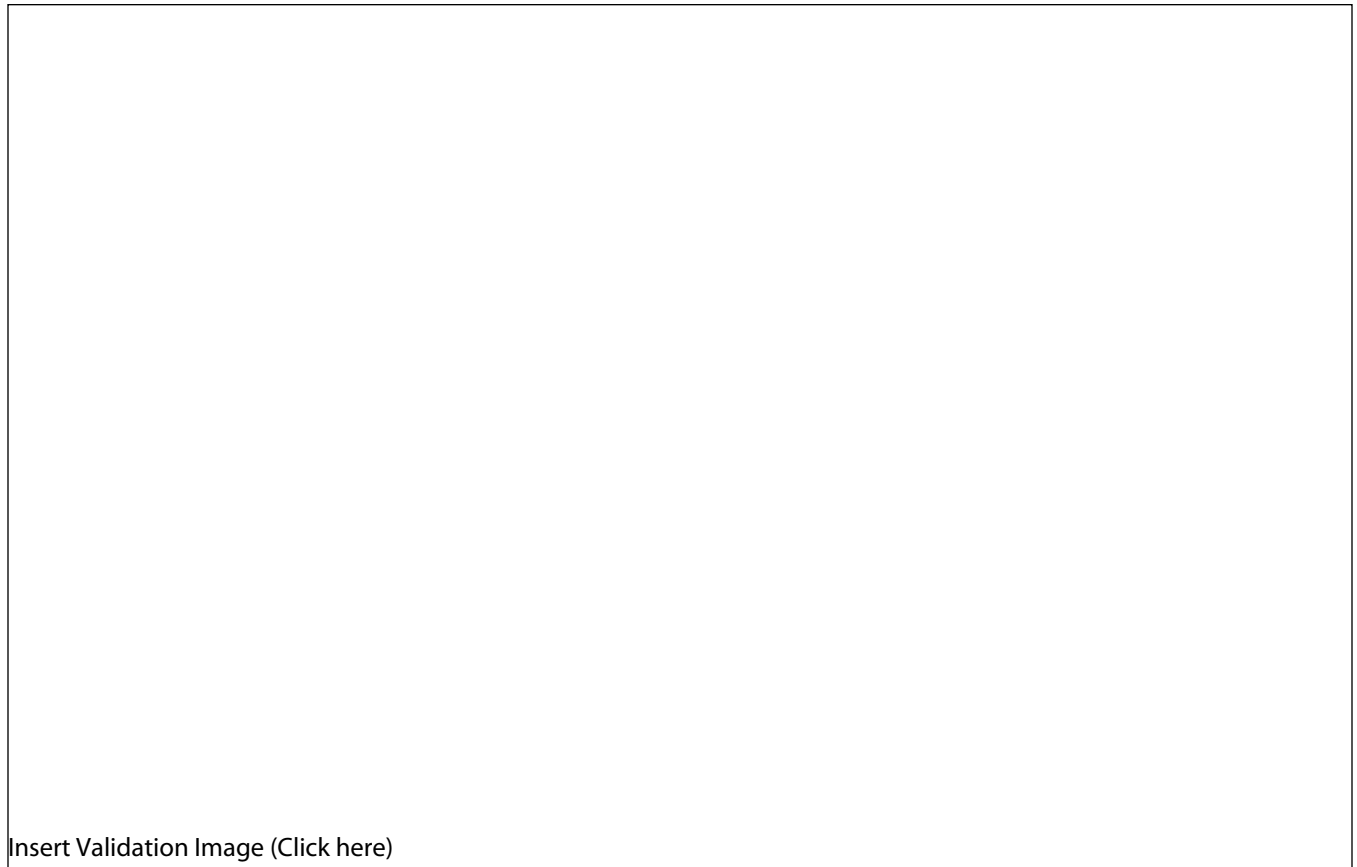
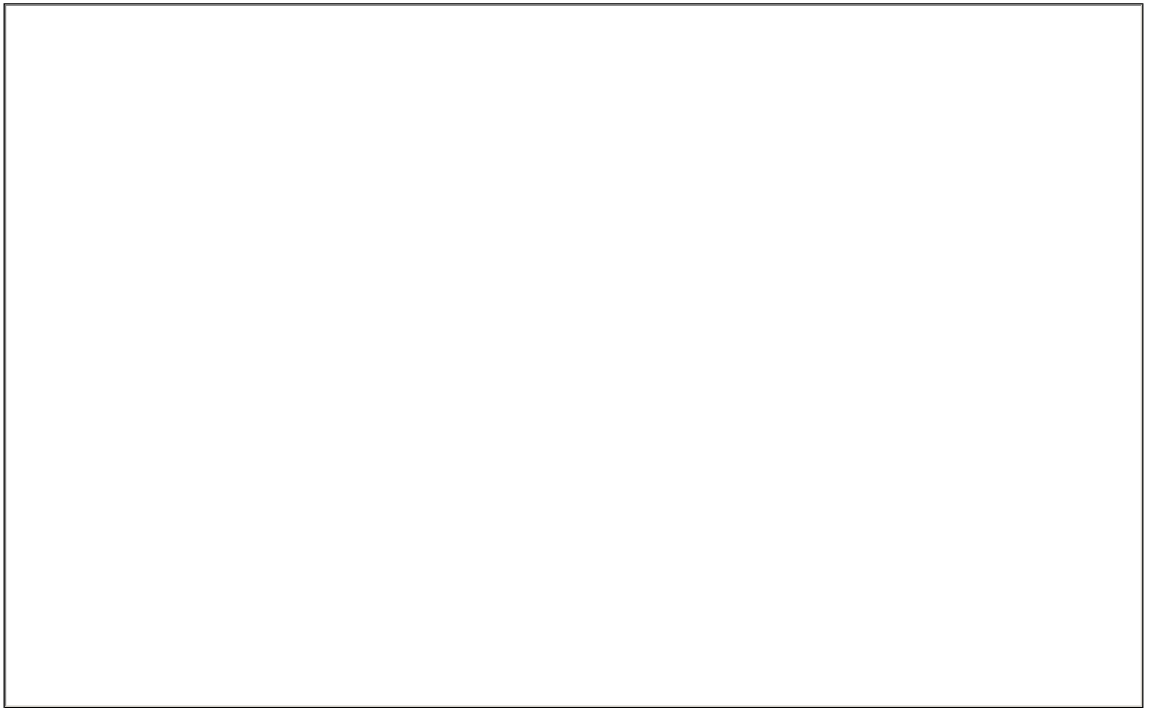
Validation #1
Analysis

Dr. Brad Bernstein and his colleagues at the Broad Institute have already validated this and several other antibodies directed against specific histone modifications. They spotted synthetic peptides containing one of about 20 histone modifications on a blot, in two different concentrations. The blot was then allowed to react with the antibody, and the antigen-antibody complexes were visualized and quantified. In each case, the antibody showed strong specificity. This is far better than showing a single band on a Western blot, since all the modifications we examine are on histone H3, and they all will show the H3 band. The Western blot will not demonstrate specificity for a particular modification, whereas the dot blot does. The relevant document for H3K9me3 is http://hgwdev.cse.ucsc.edu/ENCODE/validation/antibodies/human_H3K9me3_validation_Bernstein.pdf

Our “validation point” for mouse is that there is nothing species-specific about the existing validations. Synthetic peptides were used on the blot, and the assay was for specific reaction with the antibody. The peptides on the blot were not species-specific because HUMAN AND MOUSE HISTONE H3 ARE IDENTICAL IN THE RELEVANT REGIONS. Human and mouse H3 differ at only one position, amino acid 97, where a Cys in human is replaced by a Ser in mouse. There are NO differences in the relevant region, which is the N-terminal 36 amino acids.

Insert Validation Image (click here)

Validation #2
Analysis



Insert Validation Image (Click here)

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