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
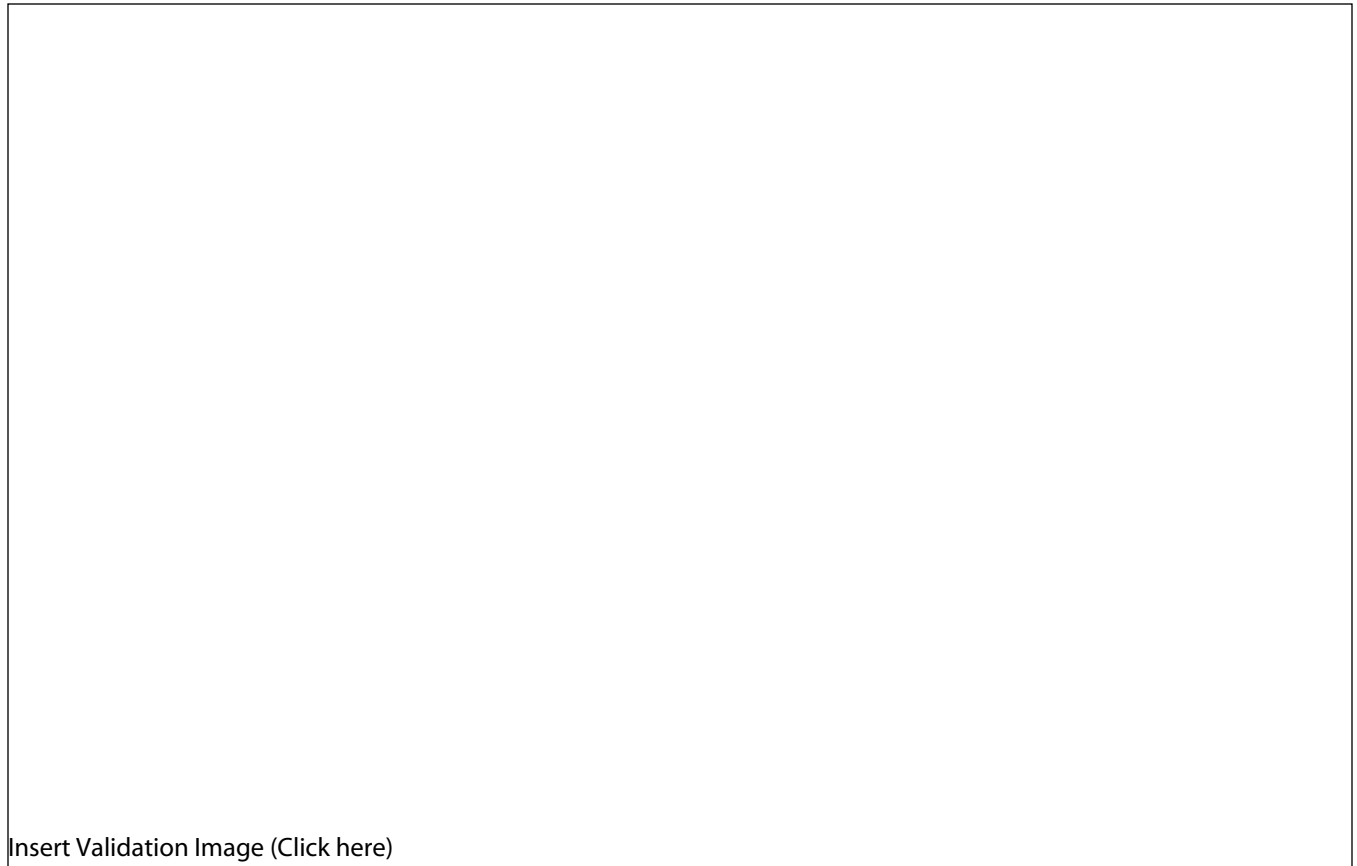
AA Position

Modification

Validation #1
Analysis

Insert Validation Image (click here)

Validation #2
Analysis

Insert Validation Image (Click here)

Validation 2: Mass Spectrometry Analysis

ENCODE data standards recognizes various methodologies for secondary validation of antibodies. Among these methodologies is immunoprecipitation followed by mass spectrometry analysis. Briefly, GM12878 nuclear extract was immunoprecipitated using primary antibody, and the IP fraction was loaded on a 12% acrylamide gel and separated with a Bio-Rad PROTEAN II xi system. Gel was stained with Coomasie Blue in order to visualize marker bands. A gel fragment corresponding to the band indicated above in the western blot image was excised and sent to the University of Alabama at Birmingham Cancer Center Mass Spectrometry/Proteomics Shared Facility. There the sample was run on an LTQ XL Linear Ion Trap Mass Spectrometer with alternating collision-induced dissociation and electron-transfer dissociation. Peptides were identified using MASCOT (Matrix Science), with probability based matching at $p < 0.05$. Subsequent analysis was performed in Scaffold (Proteome Software, Inc.) at 0.0% protein FDR and 0.0% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

1. Nucleoporin p54 OS=Homo sapiens GN=NUP54 PE=1 SV=2   NUP54_HUMAN

2. 60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2 CH60_HUMAN

3. Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1 OS=Homo sapiens GN=BAIAP2L1 PE=1 SV=2 BI2L1_HUMAN

4. Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM2 PE=1 SV=4KPYM_HUMAN

5. Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1   TBA1B_HUMAN

6. Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK PE=1 SV=1 HNRPK_HUMAN

7. Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2   IGHA1_HUMAN (+1)

8. **POU domain, class 2, transcription factor 2 OS=Homo sapiens GN=POU2F2 PE=1 SV=3 PO2F2_HUMAN (+2)**