

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*

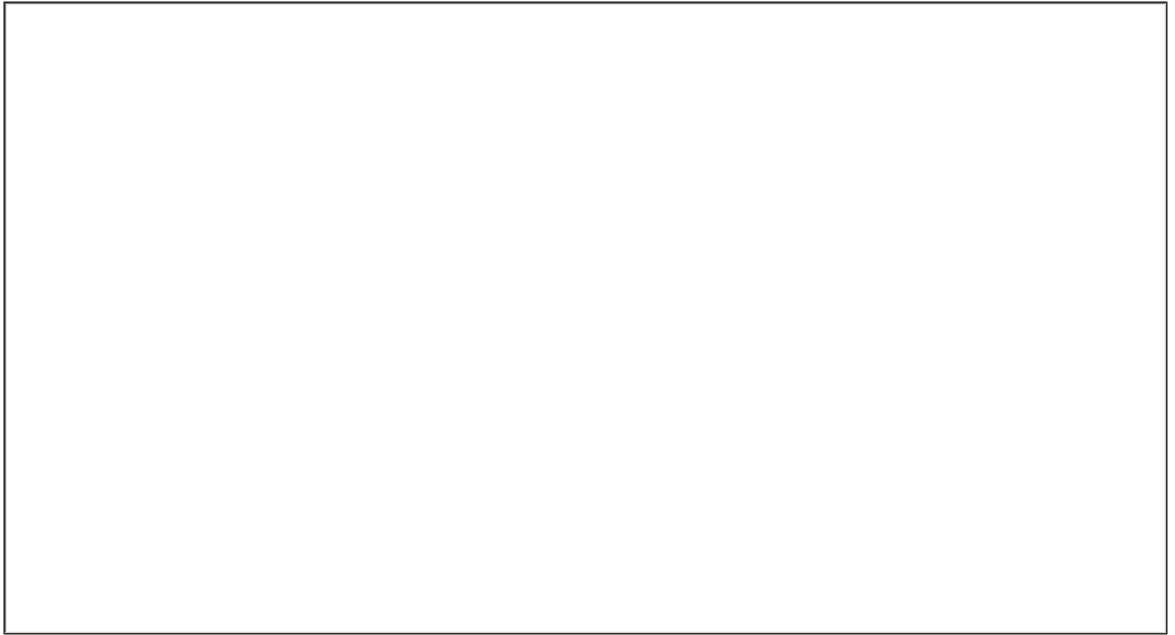
Histone Name

AA modified

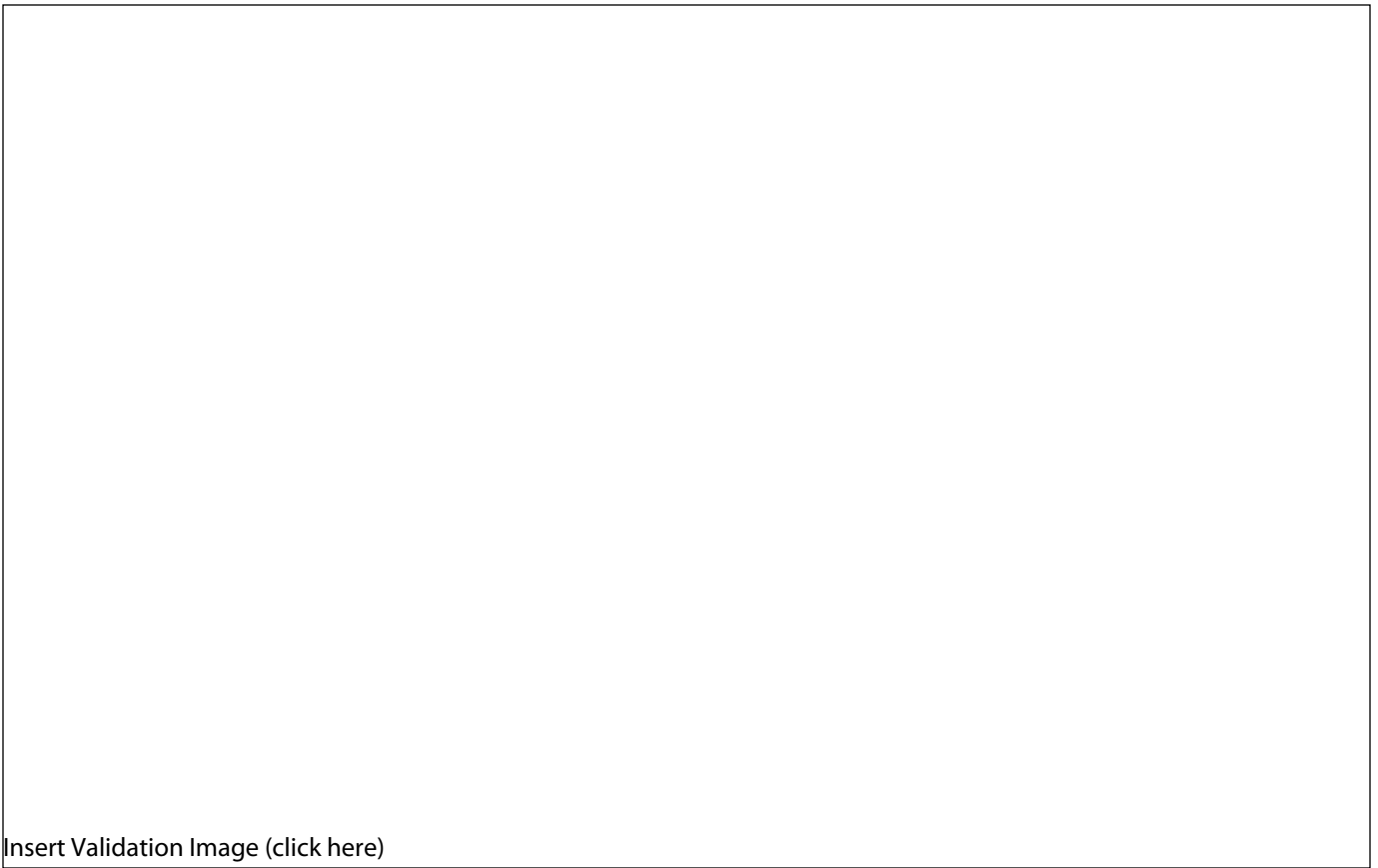
AA Position

Modification

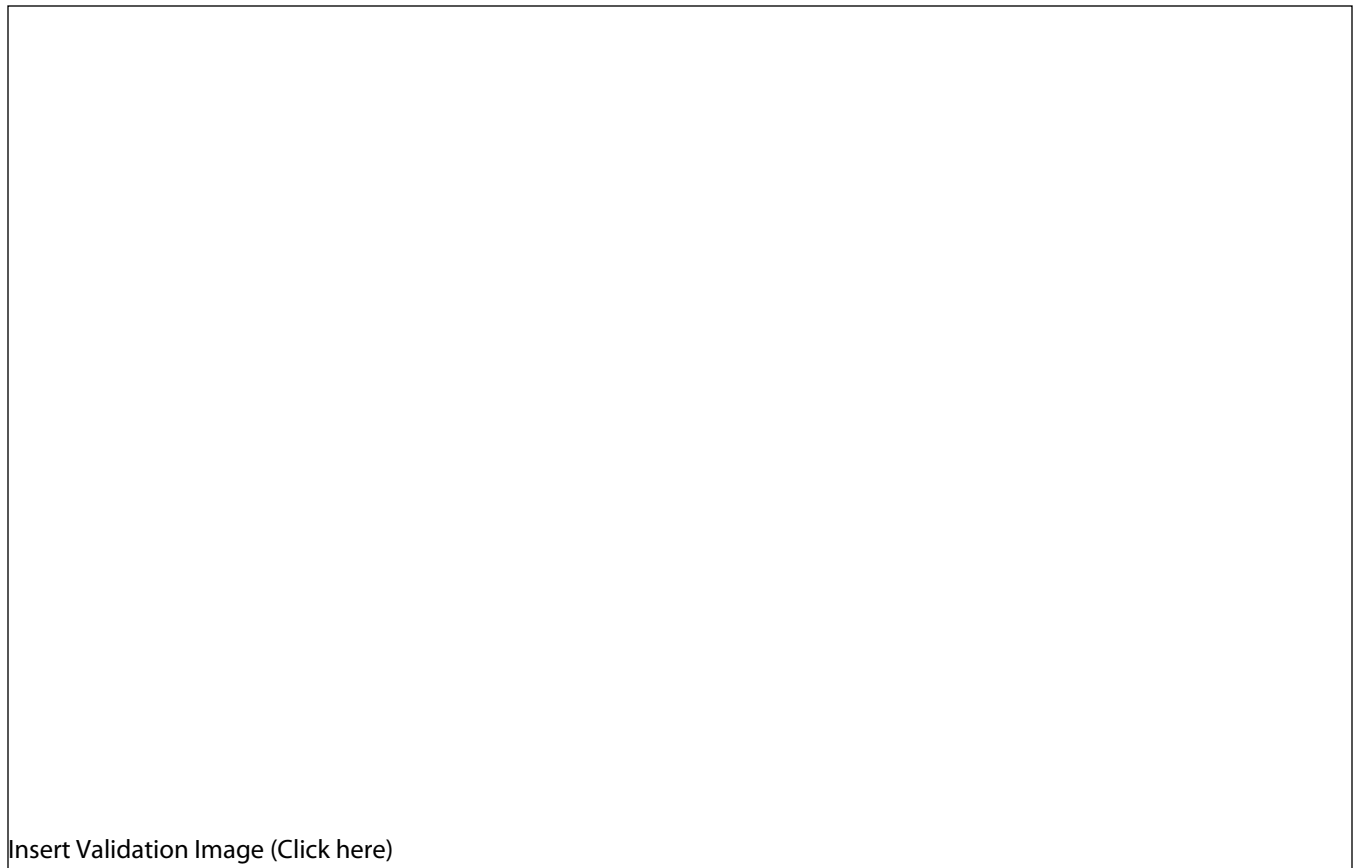
Validation #1
Analysis



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, K562 whole cell lysates were 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 Coomassie 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. Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4
HS90B_HUMAN
2. Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 K2C1_HUMAN
3. Nucleolar RNA helicase 2 OS=Homo sapiens GN=DDX21 PE=1 SV=5 DDX21_HUMAN
4. Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5
HS90A_HUMAN
5. Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6 K1C10_HUMAN
6. Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3 K1C9_HUMAN
7. Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2
K22E_HUMAN
8. DNA replication licensing factor MCM5 OS=Homo sapiens GN=MCM5 PE=1 SV=5
MCM5_HUMAN
- 9. Transcription factor 12 OS=Homo sapiens GN=TCF12 PE=1 SV=1 HTF4_HUMAN**
10. DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4
MCM7_HUMAN