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)
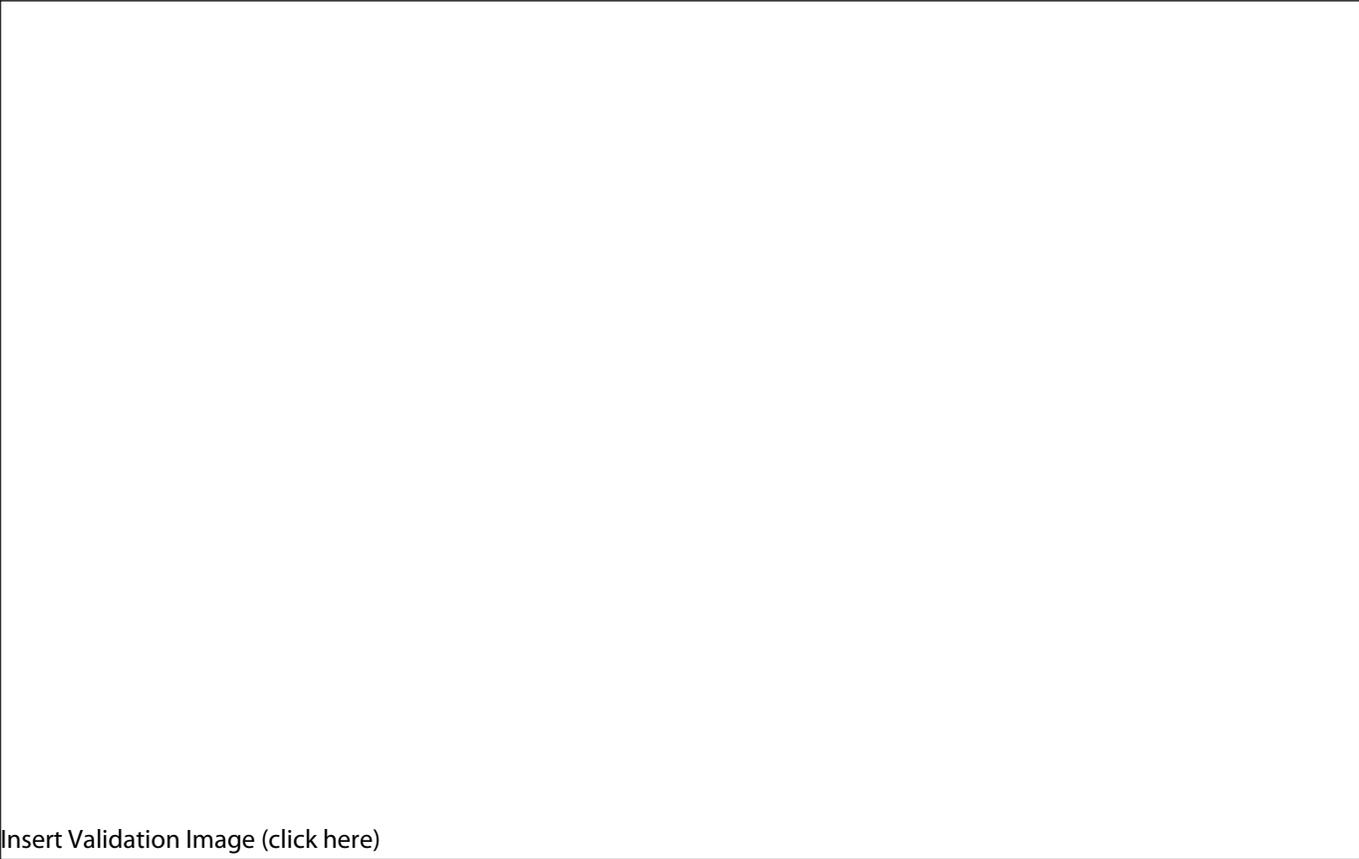
Validation #2
Analysis
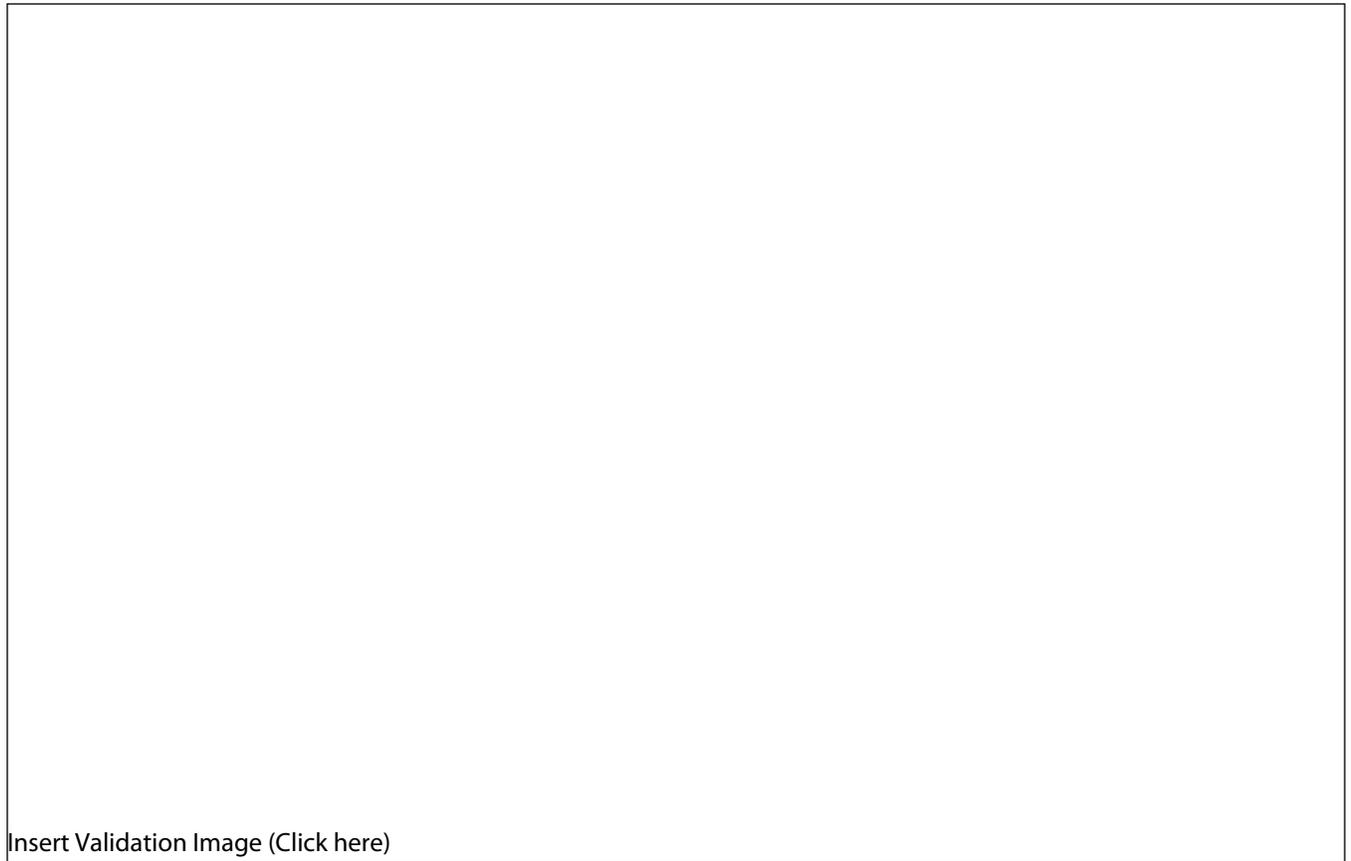
Insert Validation Image (Click here)

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 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 0.3% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

1. Actin, cytoplasmic 2, N-terminally processed n=9 Tax=Tetrapoda RepID=ACTG_HUMAN   P63261 (+6)
2. cDNA, FLJ94640, highly similar to Homo sapiens keratin 18 (KRT18), mRNA n=1 Tax=Homo sapiens RepID=B2RA03_HUMAN   B2RA03 (+1)
3. Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA_HUMAN   P06733
4. Tubulin beta chain n=12 Tax=Amniota RepID=TBB5_HUMAN   P07437
5. Cytochrome b-c1 complex subunit 2, mitochondrial n=1 Tax=Homo sapiens RepID=QCR2_HUMAN   P22695
6. Elongation factor 1-alpha 1 n=13 Tax=Eutheria RepID=EF1A1_HUMAN   P68104 (+7)
7. **Pituitary homeobox 1 n=2 Tax=Homo sapiens RepID=PITX1_HUMAN   P78337**
8. Phosphoglycerate kinase 1 n=3 Tax=Homininae RepID=PGK1_HUMAN   P00558 (+1)
9. 52 kDa Ro protein n=1 Tax=Homo sapiens RepID=RO52_HUMAN   P19474
10. 60S ribosomal protein L4 n=1 Tax=Homo sapiens RepID=RL4_HUMAN   P36578 (+2)