

# 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*

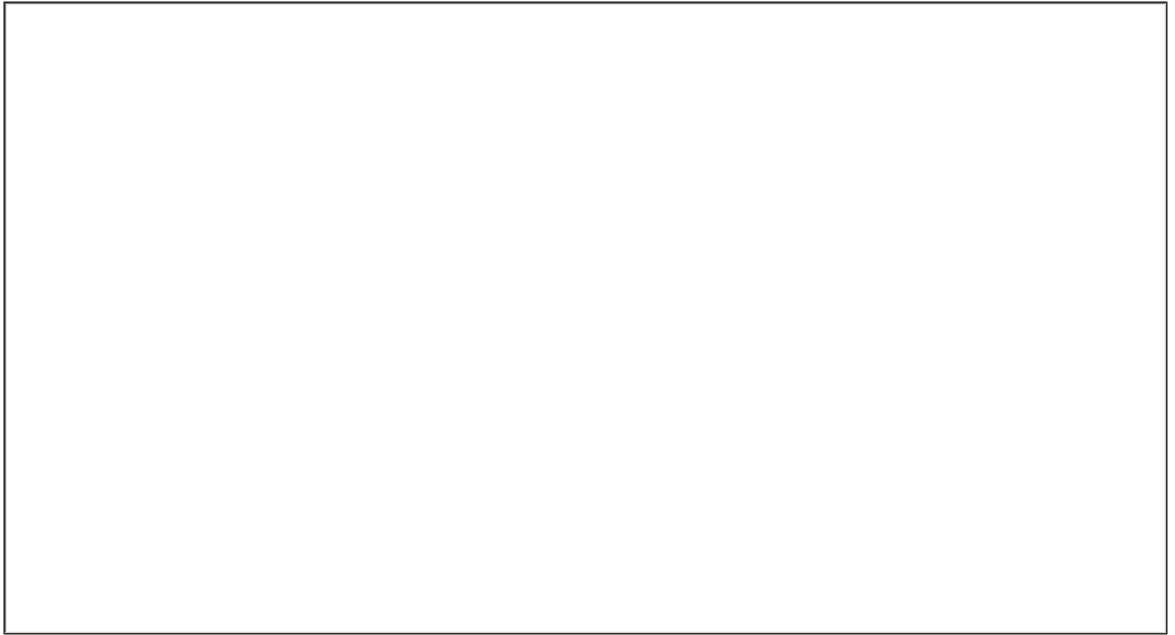
Histone Name

AA modified

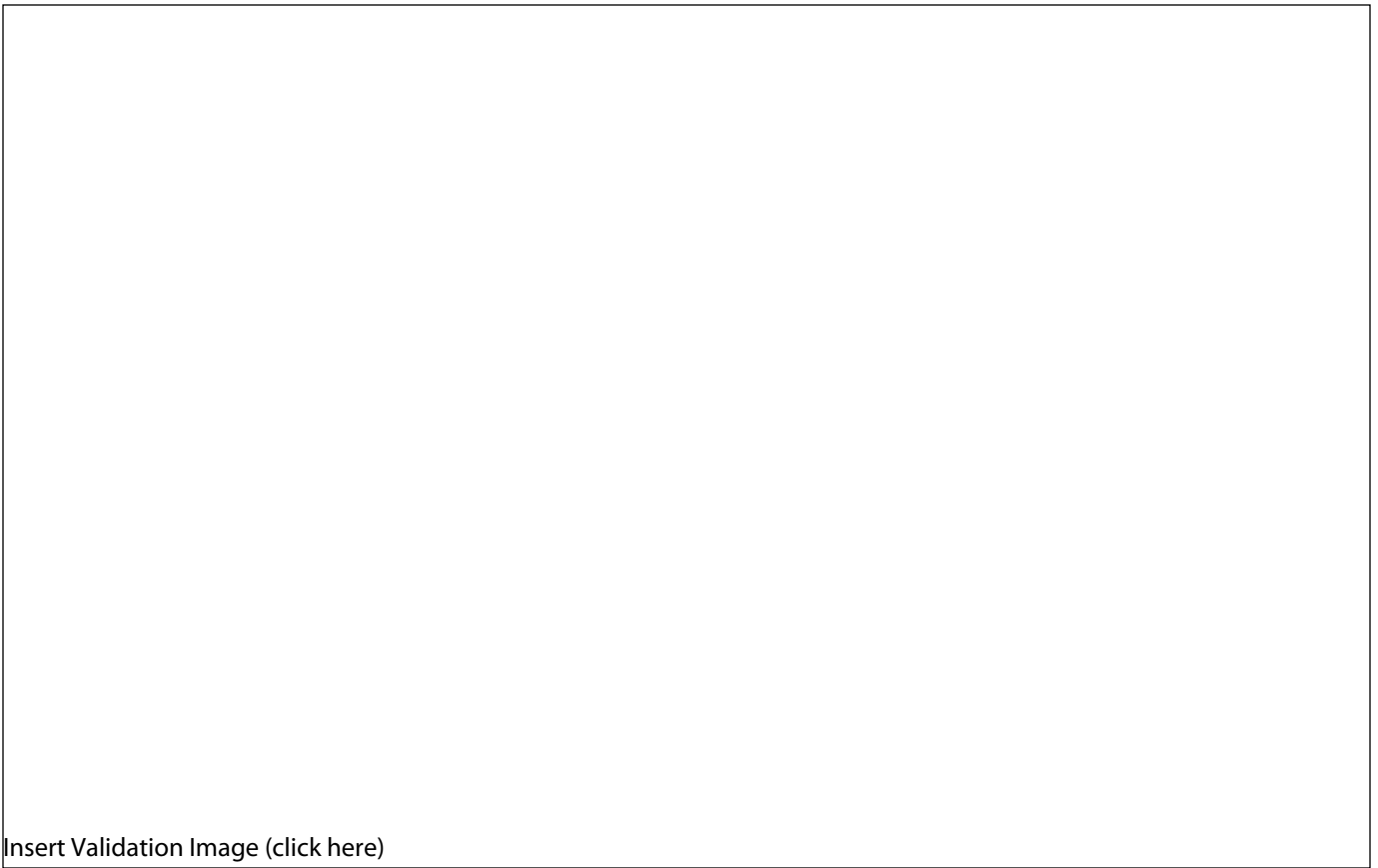
AA Position

Modification

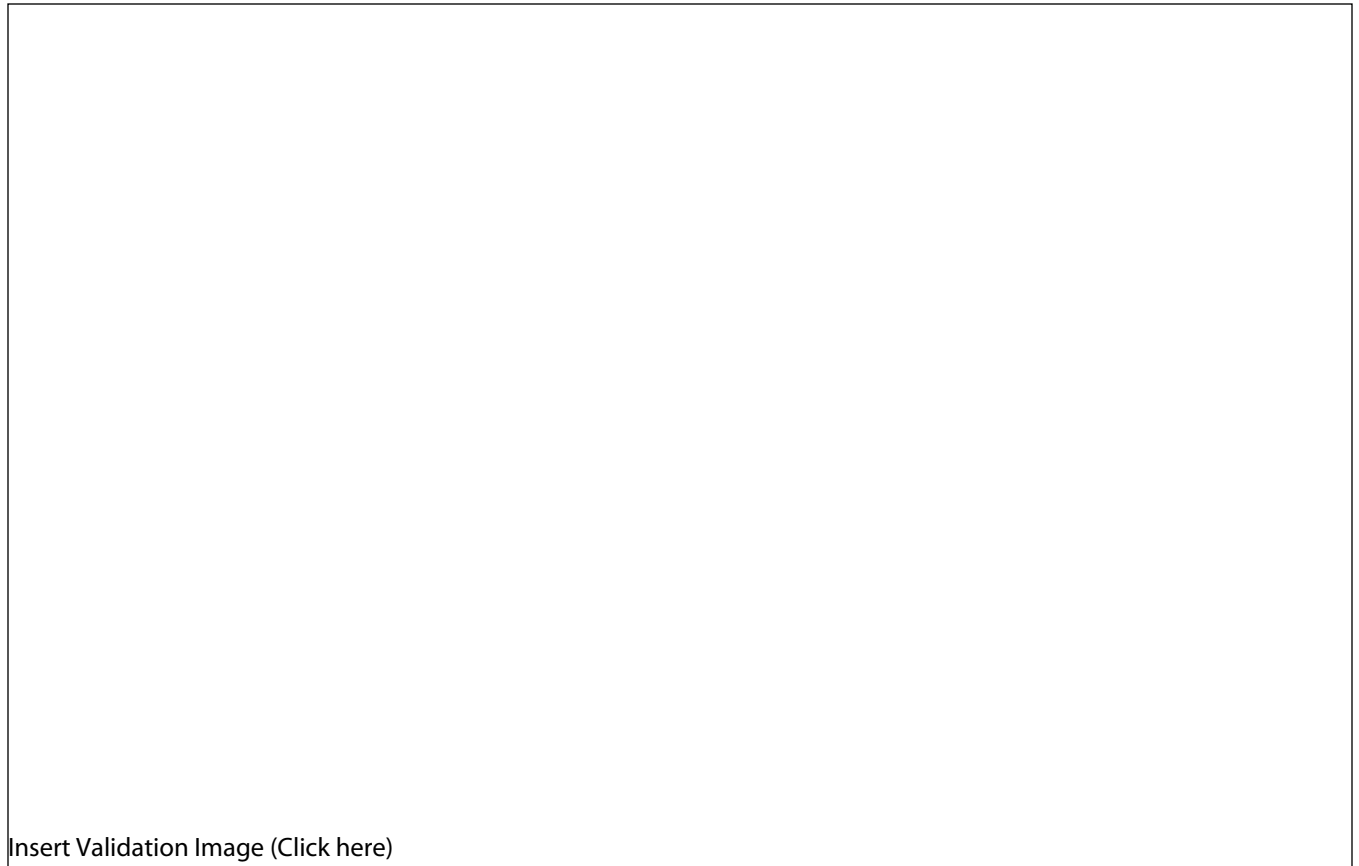
Validation #1  
Analysis



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 cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1 HSP7C\_HUMAN
2. Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5 HSP71\_HUMAN
3. T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4 TCPG\_HUMAN
4. Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 K2C1\_HUMAN
5. X-ray repair cross-complementing protein 6 OS=Homo sapiens GN=XRCC6 PE=1 SV=2 XRCC6\_HUMAN
6. Insulin-like growth factor 2 mRNA-binding protein 1 OS=Homo sapiens GN=IGF2BP1 PE=1 SV=2  
IF2B1\_HUMAN
7. Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3 HNRPM\_HUMAN
8. Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1 DDX5\_HUMAN
9. Arginyl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=RARS PE=1 SV=2 SYRC\_HUMAN
10. Eukaryotic translation initiation factor 3 subunit L OS=Homo sapiens GN=EIF3L PE=1 SV=1 EIF3L\_HUMAN
11. Phenylalanyl-tRNA synthetase beta chain OS=Homo sapiens GN=FARSB PE=1 SV=3 SYFB\_HUMAN
12. Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2 GRP75\_HUMAN
13. Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3TKT\_HUMAN
14. Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3 K1C9\_HUMAN
15. Fermitin family homolog 3 OS=Homo sapiens GN=FERMT3 PE=1 SV=1 URP2\_HUMAN
16. Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 HS90B\_HUMAN
17. Insulin-like growth factor 2 mRNA-binding protein 3 OS=Homo sapiens GN=IGF2BP3 PE=1 SV=2  
IF2B3\_HUMAN
18. Lamin A/C OS=Homo sapiens GN=LMNA PE=1 SV=1 LMNA\_HUMAN
19. Nucleolar protein 56 OS=Homo sapiens GN=NOP56 PE=1 SV=4 NOP56\_HUMAN
20. Nuclear pore complex protein Nup85 OS=Homo sapiens GN=NUP85 PE=1 SV=1 NUP85\_HUMAN
21. Calcium-binding mitochondrial carrier protein Aralar2 OS=Homo sapiens GN=SLC25A13 PE=1 SV=2  
CMC2\_HUMAN
22. Phosphatidylinositol-binding clathrin assembly protein OS=Homo sapiens GN=PICALM PE=1 SV=2  
PICAL\_HUMAN
23. Apoptosis-inducing factor 1, mitochondrial OS=Homo sapiens GN=AIFM1 PE=1 SV=1 AIFM1\_HUMAN

24. Protein arginine N-methyltransferase 5 OS=Homo sapiens GN=PRMT5 PE=1 SV=4 ANM5\_HUMAN
25. Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=1 DDX17\_HUMAN
26. Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6 K1C10\_HUMAN
27. Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2 K22E\_HUMAN
28. Phosphoenolpyruvate carboxykinase [GTP], mitochondrial OS=Homo sapiens GN=PCK2 PE=1 SV=3  
PCKGM\_HUMAN
29. Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Homo sapiens GN=RPN1 PE=1 SV=1  
RPN1\_HUMAN
30. ATP-binding cassette sub-family E member 1 OS=Homo sapiens GN=ABCE1 PE=1 SV=1 ABCE1\_HUMAN
31. Nucleolar RNA helicase 2 OS=Homo sapiens GN=DDX21 PE=1 SV=5 DDX21\_HUMAN
32. ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=3 DDX3X\_HUMAN
33. Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4 EF2\_HUMAN
34. Lamin-B1 OS=Homo sapiens GN=LMNB1 PE=1 SV=2 LMNB1\_HUMAN
35. Sec1 family domain-containing protein 1 OS=Homo sapiens GN=SCFD1 PE=1 SV=4 SCFD1\_HUMAN
36. ATPase family AAA domain-containing protein 3A OS=Homo sapiens GN=ATAD3A PE=1 SV=2 ATD3A\_HUMAN
37. Probable ATP-dependent RNA helicase DDX52 OS=Homo sapiens GN=DDX52 PE=1 SV=3 DDX52\_HUMAN
38. Nuclear RNA export factor 1 OS=Homo sapiens GN=NXF1 PE=1 SV=1 NXF1\_HUMAN
39. RNA-binding protein 39 OS=Homo sapiens GN=RBM39 PE=1 SV=2 RBM39\_HUMAN
40. Signal recognition particle 68 kDa protein OS=Homo sapiens GN=SRP68 PE=1 SV=2 SRP68\_HUMAN
41. Aspartyl-tRNA synthetase, mitochondrial OS=Homo sapiens GN=DARS2 PE=1 SV=1 SYDM\_HUMAN
42. WD repeat-containing protein 46 OS=Homo sapiens GN=WDR46 PE=1 SV=2 WDR46\_HUMAN
43. Pumilio domain-containing protein KIAA0020 OS=Homo sapiens GN=KIAA0020 PE=1 SV=3 K0020\_HUMAN
44. Leucine-rich repeat-containing protein 47 OS=Homo sapiens GN=LRRC47 PE=1 SV=1 LRC47\_HUMAN
45. Dihydropolyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial OS=Homo sapiens GN=DLAT PE=1 SV=3 ODP2\_HUMAN
46. Syntaxin-binding protein 2 OS=Homo sapiens GN=STXBP2 PE=1 SV=1 STXB2\_HUMAN
47. E3 ubiquitin/ISG15 ligase TRIM25 OS=Homo sapiens GN=TRIM25 PE=1 SV=1 TRI25\_HUMAN
48. **Zinc finger and BTB domain-containing protein 7A OS=Homo sapiens GN=ZBTB7A PE=1 SV=1  
ZBT7A\_HUMAN**