

# 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  $p < 0.05$ . As per ENCODE data standards, all MASCOT results are listed below, including common contaminants. Target protein is highlighted in bold font.

1. K2C1\_HUMAN Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6
2. K1C9\_HUMAN Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3
3. ALDOA\_HUMAN Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2
4. K1C14\_HUMAN Keratin, type I cytoskeletal 14 OS=Homo sapiens GN=KRT14 PE=1 SV=4
5. K2C6C\_HUMAN Keratin, type II cytoskeletal 6C OS=Homo sapiens GN=KRT6C PE=1 SV=3
6. K2C6B\_HUMAN Keratin, type II cytoskeletal 6B OS=Homo sapiens GN=KRT6B PE=1 SV=5
7. K1C10\_HUMAN Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6
8. PCBP1\_HUMAN Poly(rC)-binding protein 1 OS=Homo sapiens GN=PCBP1 PE=1 SV=2
9. K2C5\_HUMAN Keratin, type II cytoskeletal 5 OS=Homo sapiens GN=KRT5 PE=1 SV=3
10. K22E\_HUMAN Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2
11. K1C17\_HUMAN Keratin, type I cytoskeletal 17 OS=Homo sapiens GN=KRT17 PE=1 SV=2
12. PCBP2\_HUMAN Poly(rC)-binding protein 2 OS=Homo sapiens GN=PCBP2 PE=1 SV=1
13. HS90B\_HUMAN Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4
14. BACH\_HUMAN Cytosolic acyl coenzyme A thioester hydrolase OS=Homo sapiens GN=ACOT7 PE=1 SV=3
15. EIF3M\_HUMAN Eukaryotic translation initiation factor 3 subunit M OS=Homo sapiens GN=EIF3M PE=1 SV=1
16. TBB5\_HUMAN Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2
17. K1C16\_HUMAN Keratin, type I cytoskeletal 16 OS=Homo sapiens GN=KRT16 PE=1 SV=4
18. SPB9\_HUMAN Serpin B9 OS=Homo sapiens GN=SERPINB9 PE=1 SV=1
19. HNRPC\_HUMAN Heterogeneous nuclear ribonucleoproteins C1/C2 OS=Homo sapiens GN=HNRNPC PE=1 SV=4
20. TIM50\_HUMAN Mitochondrial import inner membrane translocase subunit TIM50 OS=Homo sapiens GN=TIMM50 PE=1 SV=2
21. NPM\_HUMAN Nucleophosmin OS=Homo sapiens GN=NPM1 PE=1 SV=2
22. AIMP2\_HUMAN Aminoacyl tRNA synthase complex-interacting multifunctional protein 2 OS=Homo sapiens GN=AIMP2 PE=1 SV=2
23. CRKL\_HUMAN Crk-like protein OS=Homo sapiens GN=CRKL PE=1 SV=1
24. H2AY\_HUMAN Core histone macro-H2A.1 OS=Homo sapiens GN=H2AFY PE=1 SV=4
25. BIEA\_HUMAN Biliverdin reductase A OS=Homo sapiens GN=BLVRA PE=1 SV=2
26. RL4\_HUMAN 60S ribosomal protein L4 OS=Homo sapiens GN=RPL4 PE=1 SV=5
27. HS90A\_HUMAN Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5
28. IF2A\_HUMAN Eukaryotic translation initiation factor 2 subunit 1 OS=Homo sapiens GN=EIF2S1 PE=1 SV=3
29. RT05\_HUMAN 28S ribosomal protein S5, mitochondrial OS=Homo sapiens GN=MRPS5 PE=1 SV=2
30. RLA0\_HUMAN 60S acidic ribosomal protein P0 OS=Homo sapiens GN=RPLP0 PE=1 SV=1
31. K1C13\_HUMAN Keratin, type I cytoskeletal 13 OS=Homo sapiens GN=KRT13 PE=1 SV=4
32. SERC\_HUMAN Phosphoserine aminotransferase OS=Homo sapiens GN=PSAT1 PE=1 SV=2
33. SAE1\_HUMAN SUMO-activating enzyme subunit 1 OS=Homo sapiens GN=SAE1 PE=1 SV=1
34. KAPCA\_HUMAN cAMP-dependent protein kinase catalytic subunit alpha OS=Homo sapiens GN=PRKACA PE=1 SV=2
35. K1C19\_HUMAN Keratin, type I cytoskeletal 19 OS=Homo sapiens GN=KRT19 PE=1 SV=3
36. ENOA\_HUMAN Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2
37. TM173\_HUMAN Transmembrane protein 173 OS=Homo sapiens GN=TMEM173 PE=1 SV=1
38. GNAI2\_HUMAN Guanine nucleotide-binding protein G(i) subunit alpha-2 OS=Homo sapiens GN=GNAI2 PE=1 SV=3
39. VP26A\_HUMAN Vacuolar protein sorting-associated protein 26A OS=Homo sapiens GN=VPS26A PE=1 SV=2
40. EF1A1\_HUMAN Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1
41. EIF3I\_HUMAN Eukaryotic translation initiation factor 3 subunit I OS=Homo sapiens GN=EIF3I PE=1 SV=1
42. RFC2\_HUMAN Replication factor C subunit 2 OS=Homo sapiens GN=RFC2 PE=1 SV=3
43. GNA13\_HUMAN Guanine nucleotide-binding protein subunit alpha-13 OS=Homo sapiens GN=GNA13 PE=1 SV=2

44. GNAI1\_HUMAN Guanine nucleotide-binding protein G(i) subunit alpha-1 OS=Homo sapiens GN=GNAI1 PE=1 SV=2

45. K1C24\_HUMAN Keratin, type I cytoskeletal 24 OS=Homo sapiens GN=KRT24 PE=1 SV=1

46. ADHX\_HUMAN Alcohol dehydrogenase class-3 OS=Homo sapiens GN=ADH5 PE=1 SV=4

47. TWF2\_HUMAN Twinfilin-2 OS=Homo sapiens GN=TWF2 PE=1 SV=2

48. K2C1B\_HUMAN Keratin, type II cytoskeletal 1b OS=Homo sapiens GN=KRT77 PE=1 SV=2

49. KT222\_HUMAN Keratin-like protein KRT222 OS=Homo sapiens GN=KRT222 PE=2 SV=1

50. ATPB\_HUMAN ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3

51. LDHB\_HUMAN L-lactate dehydrogenase B chain OS=Homo sapiens GN=LDHB PE=1 SV=2

52. ERLN1\_HUMAN Erlin-1 OS=Homo sapiens GN=ERLIN1 PE=1 SV=1

53. ALDOC\_HUMAN Fructose-bisphosphate aldolase C OS=Homo sapiens GN=ALDOC PE=1 SV=2

54. LDHC\_HUMAN L-lactate dehydrogenase C chain OS=Homo sapiens GN=LDHC PE=2 SV=4

55. BUB3\_HUMAN Mitotic checkpoint protein BUB3 OS=Homo sapiens GN=BUB3 PE=1 SV=1

56. NSDHL\_HUMAN Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating OS=Homo sapiens GN=NSDHL PE=1 SV=2

57. TBA1B\_HUMAN Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1

58. RRP7A\_HUMAN Ribosomal RNA-processing protein 7 homolog A OS=Homo sapiens GN=RRP7A PE=1 SV=2

59. EIF2BB\_HUMAN Translation initiation factor eIF-2B subunit beta OS=Homo sapiens GN=EIF2B2 PE=1 SV=3

60. **SPI1\_HUMAN Transcription factor PU.1 OS=Homo sapiens GN=SPI1 PE=1 SV=2**

61. HNRCL\_HUMAN Heterogeneous nuclear ribonucleoprotein C-like 1 OS=Homo sapiens GN=HNRNPCL1 PE=1 SV=1

62. TRAP1\_HUMAN Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3

63. K2C73\_HUMAN Keratin, type II cytoskeletal 73 OS=Homo sapiens GN=KRT73 PE=1 SV=1

64. TOPK\_HUMAN Lymphokine-activated killer T-cell-originated protein kinase OS=Homo sapiens GN=PBK PE=1 SV=3

65. K1C28\_HUMAN Keratin, type I cytoskeletal 28 OS=Homo sapiens GN=KRT28 PE=1 SV=2

66. THIL\_HUMAN Acetyl-CoA acetyltransferase, mitochondrial OS=Homo sapiens GN=ACAT1 PE=1 SV=1

67. REV\_5682

68. AHSÄ1\_HUMAN Activator of 90 kDa heat shock protein ATPase homolog 1 OS=Homo sapiens GN=AHSÄ1 PE=1 SV=1

69. K1C12\_HUMAN Keratin, type I cytoskeletal 12 OS=Homo sapiens GN=KRT12 PE=1 SV=1

70. AK1C2\_HUMAN Aldo-keto reductase family 1 member C2 OS=Homo sapiens GN=AKR1C2 PE=1 SV=3

71. FPPS\_HUMAN Farnesyl pyrophosphate synthase OS=Homo sapiens GN=FDPS PE=1 SV=4

72. AK1C1\_HUMAN Aldo-keto reductase family 1 member C1 OS=Homo sapiens GN=AKR1C1 PE=1 SV=1

73. RAE1L\_HUMAN mRNA export factor OS=Homo sapiens GN=RAE1 PE=1 SV=1

74. SET\_HUMAN Protein SET OS=Homo sapiens GN=SET PE=1 SV=3

75. K1C26\_HUMAN Keratin, type I cytoskeletal 26 OS=Homo sapiens GN=KRT26 PE=1 SV=2

76. REV\_11034

77. NDUÄÄ\_HUMAN NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial OS=Homo sapiens GN=NDUÄÄ10 PE=1 SV=1

78. PSD7\_HUMAN 26S proteasome non-ATPase regulatory subunit 7 OS=Homo sapiens GN=PSMD7 PE=1 SV=2

79. MK01\_HUMAN Mitogen-activated protein kinase 1 OS=Homo sapiens GN=MAPK1 PE=1 SV=3

80. MK03\_HUMAN Mitogen-activated protein kinase 3 OS=Homo sapiens GN=MAPK3 PE=1 SV=4

81. BACHL\_HUMAN Cytosolic acyl coenzyme A thioester hydrolase-like OS=Homo sapiens GN=ACOT7L PE=1 SV=1

82. IGHG4\_HUMAN Ig gamma-4 chain C region OS=Homo sapiens GN=IGHG4 PE=1 SV=1

83. DYH7\_HUMAN Dynein heavy chain 7, axonemal OS=Homo sapiens GN=DNAH7 PE=1 SV=1

84. LIMA1\_HUMAN LIM domain and actin-binding protein 1 OS=Homo sapiens GN=LIMA1 PE=1 SV=1

85. KIF5C\_HUMAN Kinesin heavy chain isoform 5C OS=Homo sapiens GN=KIF5C PE=1 SV=1

86. DESP\_HUMAN Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3

87. STRAP\_HUMAN Serine-threonine kinase receptor-associated protein OS=Homo sapiens GN=STRAP PE=1 SV=1