ENCODE DCC Antibody Validation Document

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| Validation Method #1 Validation Method #2 |
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| Purification Polyclonal/ |
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| Validation #1 Analysis | | |
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| Validation #2 Analysis | | | | |
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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, HeLa 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. As per ENCODE data standards, all MASCOT results are listed below, including common contaminants. Target protein is highlighted in bold font.

- 1. P14618 Pyruvate kinase isozymes M1/M2 n=2 Tax=Homininae RepID=KPYM_HUMAN
- 2. P08729 Keratin, type II cytoskeletal 7 n=1 Tax=Homo sapiens RepID=K2C7 HUMAN
- 3. UPI0001AE6926 UPI0001AE6926 related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE6926
- 4. P08670 Vimentin n=2 Tax=Homo sapiens RepID=VIME_HUMAN
- 5. UPI00017BCE7F keratin, type I cytoskeletal 10 n=1 Tax=Homo sapiens RepID=UPI00017BCE7F
- 6. P17987 T-complex protein 1 subunit alpha n=2 Tax=Homininae RepID=TCPA_HUMAN
- 7. A8K3C3 T-complex protein 1 subunit delta n=1 Tax=Homo sapiens RepID=A8K3C3 HUMAN
- 8. P04264 Keratin, type II cytoskeletal 1 n=1 Tax=Homo sapiens RepID=K2C1 HUMAN
- 9. Q4R5E7 Brain cDNA, clone: QnpA-15907, similar to human non-POU domain containing, octamer-binding (NONO), n=1 Tax=Macaca fascicularis RepID=Q4R5E7_MACFA
- 10. B4DG39 Glucose-6-phosphate isomerase n=2 Tax=Homo sapiens RepID=B4DG39 HUMAN
- 11. B4DVJ0 Glucose-6-phosphate isomerase n=1 Tax=Homo sapiens RepID=B4DVJ0 HUMAN
- 12. Q99832 T-complex protein 1 subunit eta n=1 Tax=Homo sapiens RepID=TCPH HUMAN
- 13. O43175 D-3-phosphoglycerate dehydrogenase n=1 Tax=Homo sapiens RepID=SERA HUMAN
- 14. B5BTY7 T-complex protein 1 subunit beta n=1 Tax=Homo sapiens RepID=B5BTY7_HUMAN
- 15. P05787 Keratin, type II cytoskeletal 8 n=1 Tax=Homo sapiens RepID=K2C8 HUMAN
- 16. P02769 Serum albumin n=1 Tax=Bos taurus RepID=ALBU BOVIN
- 17. UPI000179EC85 Serum albumin precursor (Allergen Bos d 6) (BSA). n=1 Tax=Bos taurus RepID=UPI000179EC85
- 18. B3KPS3 cDNA FLJ32131 fis, clone PEBLM2000267, highly similar to Tubulin alphaubiquitous chain n=1 Tax=Homo sapiens RepID=B3KPS3_HUMAN
- 19. P35908 Keratin, type II cytoskeletal 2 epidermal n=1 Tax=Homo sapiens RepID=K22E_HUMAN
- 20. B0YIW6 Archain 1, isoform CRA_a n=1 Tax=Homo sapiens RepID=B0YIW6_HUMAN
- 21. Q6DD88 Atlastin-3 n=1 Tax=Homo sapiens RepID=ATLA3 HUMAN
- 22. B4DNL5 cDNA FLJ59361, highly similar to Protein disulfide-isomerase (EC 5.3.4.1) n=1 Tax=Homo sapiens RepID=B4DNL5 HUMAN
- 23. UPI000059DB4B UPI000059DB4B related cluster n=1 Tax=Homo sapiens RepID=UPI000059DB4B
- 24. P35527 Keratin, type I cytoskeletal 9 n=1 Tax=Homo sapiens RepID=K1C9 HUMAN
- 25. Q9BUQ0 Polypyrimidine tract binding protein 1 n=1 Tax=Homo sapiens RepID=Q9BUQ0 HUMAN

- 26. P40227 T-complex protein 1 subunit zeta n=1 Tax=Homo sapiens RepID=TCPZ HUMAN
- 27. C5IWV5 Trypsingen n=1 Tax=Sus scrofa RepID=C5IWV5 PIG
- 28. P08243 Asparagine synthetase [glutamine-hydrolyzing] n=2 Tax=Homo sapiens RepID=ASNS HUMAN
- 29. B3KWQ6 cDNA FLJ43599 fis, clone SMINT2017781, highly similar to PERIPHERIN n=1 Tax=Homo sapiens RepID=B3KWQ6 HUMAN
- P04259 Keratin, type II cytoskeletal 6B n=1 Tax=Homo sapiens RepID=K2C6B HUMAN
- 31. Q2Q9H2 Glucose-6-phosphate 1-dehydrogenase (Fragment) n=1 Tax=Homo sapiens RepID=Q2Q9H2 HUMAN
- 32. GB0001 Retinoid X Receptor, alpha, per hprd
- 33. Q5XKE5 Keratin, type II cytoskeletal 79 n=1 Tax=Homo sapiens RepID=K2C79 HUMAN
- 34. O95747 Serine/threonine-protein kinase OSR1 n=1 Tax=Homo sapiens RepID=OXSR1 HUMAN
- 35. B2RBS8 cDNA, FLJ95666, highly similar to Homo sapiens albumin (ALB), mRNA n=1 Tax=Homo sapiens RepID=B2RBS8_HUMAN
- 36. UPI0001AE6B0B UPI0001AE6B0B related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE6B0B
- 37. A5Z217 Mutant desmin n=1 Tax=Homo sapiens RepID=A5Z217 HUMAN
- 38. UPI000186CFF7 T-complex protein 1 subunit alpha, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186CFF7
- 39. O95678 Keratin, type II cytoskeletal 75 n=1 Tax=Homo sapiens RepID=K2C75_HUMAN
- 40. Q14CN4 Keratin, type II cytoskeletal 72 n=1 Tax=Homo sapiens RepID=K2C72 HUMAN
- 41. A2RRD3 RBM39 protein n=1 Tax=Homo sapiens RepID=A2RRD3 HUMAN
- 42. Q0IIN1 Keratin 77 n=1 Tax=Homo sapiens RepID=Q0IIN1 HUMAN
- 43. P61626 Lysozyme C n=4 Tax=Homininae RepID=LYSC HUMAN
- 44. B4DKV4 cDNA FLJ60647, highly similar to Keratin, type II cytoskeletal 6B n=1 Tax=Homo sapiens RepID=B4DKV4 HUMAN
- 45. B4DGL0 cDNA FLJ53619, highly similar to Heat shock protein HSP 90-beta n=1 Tax=Homo sapiens RepID=B4DGL0_HUMAN
- 46. B4E1T1 cDNA FLJ54081, highly similar to Keratin, type II cytoskeletal 5 n=1 Tax=Homo sapiens RepID=B4E1T1_HUMAN
- 47. Q7Z2V5 Putative uncharacterized protein DKFZp686J01190 (Fragment) n=1 Tax=Homo sapiens RepID=Q7Z2V5 HUMAN
- 48. Q53G99 Beta actin variant (Fragment) n=1 Tax=Homo sapiens RepID=Q53G99 HUMAN
- 49. Q4R3D5 Testis cDNA clone: QtsA-17726, similar to human keratin protein K6irs (K6IRS2), n=1 Tax=Macaca fascicularis RepID=Q4R3D5_MACFA
- 50. UPI0001D3B45B UPI0001D3B45B related cluster n=1 Tax=Homo sapiens RepID=UPI0001D3B45B
- 51. B1AHM1 DEAD (Asp-Glu-Ala-Asp) box polypeptide 17 n=1 Tax=Homo sapiens RepID=B1AHM1 HUMAN
- 52. B4DFL1 Dihydrolipoyl dehydrogenase n=2 Tax=Homo sapiens RepID=B4DFL1 HUMAN
- 53. UPI0000E4FB5A keratin, type I cytoskeletal 13 isoform a n=1 Tax=Homo sapiens RepID=UPI0000E4FB5A
- 54. A8K2H9 cDNA FLJ78503, highly similar to Homo sapiens keratin 13 (KRT13), transcript variant 1, mRNA n=1 Tax=Homo sapiens RepID=A8K2H9_HUMAN
- 55. P78386 Keratin, type II cuticular Hb5 n=1 Tax=Homo sapiens RepID=KRT85_HUMAN
- 56. B4E0S6 cDNA FLJ55635, highly similar to pre-mRNA-splicing factorATP-dependent RNA helicase DHX15 (EC 3.6.1.-) n=1 Tax=Homo sapiens RepID=B4E0S6 HUMAN
- 57. Q5LJ98 TROVE domain family, member 2 n=1 Tax=Homo sapiens RepID=Q5LJ98_HUMAN
- 58. Q5T6W1 Heterogeneous nuclear ribonucleoprotein K n=1 Tax=Homo sapiens RepID=Q5T6W1 HUMAN
- 59. Q59FN3 Acyl-Coenzyme A dehydrogenase family, member 9 variant (Fragment) n=1 Tax=Homo sapiens RepID=Q59FN3_HUMAN
- 60. UPI000186EB8A 26S protease regulatory subunit, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186EB8A

- 61. UPI000198CA76 UPI000198CA76 related cluster n=1 Tax=Homo sapiens RepID=UPI000198CA76
- 62. UPI0001AE685C UPI0001AE685C related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE685C
- 63. UPI0001AE6AEA UPI0001AE6AEA related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE6AEA
- 64. Q2L9C1 Dha kinase/FMN cyclase splice variant n=1 Tax=Homo sapiens RepID=Q2L9C1_HUMAN
- 65. UPI000186E8F0 tubulin beta-2 chain, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186E8F0
- 66. UPI0000F6E6ED Elongator complex protein 3 (EC 2.3.1.48) (hELP3). n=1 Tax=Homo sapiens RepID=UPI0000F6E6ED
- 67. P02533 Keratin, type I cytoskeletal 14 n=1 Tax=Homo sapiens RepID=K1C14_HUMAN
- 68. Q7Z3M3 Putative uncharacterized protein DKFZp686L04275 (Fragment) n=1 Tax=Homo sapiens RepID=Q7Z3M3_HUMAN
- 69. B2R9U2 cDNA, FLJ94557, highly similar to Homo sapiens FK506 binding protein 4, 59kDa (FKBP4), mRNA n=1 Tax=Homo sapiens RepID=B2R9U2_HUMAN
- 70. Q6MZW0 Putative uncharacterized protein DKFZp686J11235 (Fragment) n=1 Tax=Homo sapiens RepID=Q6MZW0 HUMAN
- 72. UPI0001AE74B4 UPI0001AE74B4 related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE74B4
- 73. B3KNX7 cDNA FLJ30666 fis, clone FCBBF1000627, highly similar to Serine/threonine-protein kinase PAK 1 (EC 2.7.11.1) n=1 Tax=Homo sapiens RepID=B3KNX7 HUMAN
- 74. B3KX11 cDNA FLJ44436 fis, clone UTERU2019706, highly similar to T-complex protein 1 subunit gamma n=1 Tax=Homo sapiens RepID=B3KX11 HUMAN
- 75. B2R7V4 cDNA, FLJ93619, highly similar to Homo sapiens PRP4 pre-mRNA processing factor 4 homolog (yeast) (PRPF4), mRNA n=1 Tax=Homo sapiens RepID=B2R7V4 HUMAN
- 76. Q4R9A4 Testis cDNA clone: QtsA-10416, similar to human DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked(DDX3X), transcript variant 1, n=1 Tax=Macaca fascicularis RepID=Q4R9A4_MACFA
- 77. P54136 Arginyl-tRNA synthetase, cytoplasmic n=2 Tax=Homo sapiens RepID=SYRC_HUMAN
- 78. B2R7P8 cDNA, FLJ93545, highly similar to Homo sapiens 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC), mRNA n=1 Tax=Homo sapiens RepID=B2R7P8 HUMAN
- 79. B6ZGT6 Retinoid X receptor-gamma n=1 Tax=Homo sapiens RepID=B6ZGT6_HUMAN
- 80. UPI000013F740 T-complex protein 1 subunit zeta-2 n=1 Tax=Homo sapiens RepID=UPI000013F740
- 81. UPI000186CBCD T-complex protein 1 subunit eta, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186CBCD
- 82. UPI000186CFD7 tubulin beta chain, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186CFD7
- 83. O75758 Pyruvate kinase n=1 Tax=Homo sapiens RepID=O75758_HUMAN
- 84. Q9BV61 TRAP1 protein (Fragment) n=1 Tax=Homo sapiens RepID=Q9BV61_HUMAN
- 85. UPI000186D735 heat shock protein 75 kDa, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186D735
- 86. P00762 Anionic trypsin-1 n=1 Tax=Rattus norvegicus RepID=TRY1_RAT
- 87. UPI0000D62052 Torsin-1A-interacting protein 1. n=1 Tax=Homo sapiens RepID=UPI0000D62052
- 88. UPI00001AECF3 UPI00001AECF3 related cluster n=1 Tax=Homo sapiens RepID=UPI00001AECF3
- 89. UPI00005E77FA PREDICTED: similar to likely orthologue of Homo sapiens tubulin beta 1 (TUBB1) n=1 Tax=Monodelphis domestica RepID=UPI00005E77FA
- 90. Q9BSV4 SFPQ protein (Fragment) n=1 Tax=Homo sapiens RepID=Q9BSV4_HUMAN

- 91. B1ALY5 ROD1 regulator of differentiation 1 (S. pombe) n=2 Tax=Homo sapiens RepID=B1ALY5 HUMAN
- 92. Q59G43 Polypyrimidine tract binding protein 2 variant (Fragment) n=1 Tax=Homo sapiens RepID=Q59G43_HUMAN
- 93. B3KWR2 cDNA FLJ43638 fis, clone SPLEN2039845, highly similar to IMPORTIN ALPHA-1 SUBUNIT n=1 Tax=Homo sapiens RepID=B3KWR2 HUMAN
- 94. B4DSS8 cDNA FLJ55936, highly similar to Polypyrimidine tract-binding protein 2 n=1 Tax=Homo sapiens RepID=B4DSS8_HUMAN
- 95. UPI0000E5924D Histone deacetylase 2 (HD2). n=1 Tax=Homo sapiens RepID=UPI0000E5924D
- 96. B7Z7J5 cDNA FLJ61673, highly similar to Retinoic acid receptor RXR-beta n=1 Tax=Homo sapiens RepID=B7Z7J5 HUMAN
- 97. Q86Y46 Keratin, type II cytoskeletal 73 n=1 Tax=Homo sapiens RepID=K2C73 HUMAN
- 98. P08779 Keratin, type I cytoskeletal 16 n=1 Tax=Homo sapiens RepID=K1C16 HUMAN
- 99. Q7Z3Y9 Keratin, type I cytoskeletal 26 n=1 Tax=Homo sapiens RepID=K1C26 HUMAN
- 100. Q4R5K5 Brain cDNA, clone: QflA-10318, similar to human tyrosyl-tRNA synthetase (YARS), n=1 Tax=Macaca fascicularis RepID=Q4R5K5_MACFA
- 101. Q8IWR8 Ribosomal protein L19 (Fragment) n=4 Tax=Mammalia RepID=Q8IWR8 HUMAN
- 102. UPI0000D9FD95 keratin, type II cytoskeletal 4 n=1 Tax=Homo sapiens RepID=UPI0000D9FD95
- 103. UPI000013EEC8 keratin, type II cytoskeletal 3 n=1 Tax=Homo sapiens RepID=UPI000013EEC8
- 104. UPI000186DA7E T-complex protein 1 subunit zeta, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186DA7E
- 105. A5JHP3 Dermcidin isoform 2 n=1 Tax=Homo sapiens RepID=A5JHP3 HUMAN
- 106. UPI000186D036 tubulin beta chain, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186D036
- 107. Q01546 Keratin, type II cytoskeletal 2 oral n=1 Tax=Homo sapiens RepID=K22O HUMAN
- 108. Q3SY84 Keratin, type II cytoskeletal 71 n=1 Tax=Homo sapiens RepID=K2C71_HUMAN
- 109. Q0PHS2 Glucose-6-phosphate dehydrogenase (Fragment) n=1 Tax=Homo sapiens RepID=Q0PHS2 HUMAN
- 110. B4DFM5 cDNA FLJ51779, highly similar to Vacuolar ATP synthase subunit B, brain isoform (EC 3.6.3.14) n=1 Tax=Homo sapiens RepID=B4DFM5_HUMAN
- 111. B0QZK4 Heterochromatin protein 1, binding protein 3 (Fragment) n=1 Tax=Homo sapiens RepID=B0QZK4 HUMAN
- 112. UPI00006C0D04 PREDICTED: similar to actin related protein 2/3 complex subunit 1B n=1 Tax=Homo sapiens RepID=UPI00006C0D04
- 113. UPI000186E7A6 T-complex protein 1 subunit beta, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186E7A6
- 114. B4DXJ7 cDNA FLJ53235, highly similar to Caseinolytic peptidase B protein n=1 Tax=Homo sapiens RepID=B4DXJ7 HUMAN