

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 1.7% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

1. T-complex protein 1 subunit alpha n=2 Tax=Homininae RepID=TCPA_HUMAN P17987
2. Pyruvate kinase isozymes M1/M2 n=2 Tax=Homininae RepID=KPYM_HUMAN P14618
3. PIG48 n=1 Tax=Homo sapiens RepID=Q2TU64_HUMAN Q2TU64
4. Mitochondrial heat shock 60kD protein 1 variant 1 n=1 Tax=Homo sapiens RepID=B3GQS7_HUMAN B3GQS7 (+1)
5. cDNA FLJ30049 fis, clone ADRGL1000033, highly similar to 26S proteasome non-ATPase regulatory subunit 3 n=1 Tax=Homo sapiens RepID=B3KNN7_HUMAN B3KNN7 (+4)
6. 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 B2R7P8 (+1)
7. cDNA FLJ54957, highly similar to Transketolase (EC 2.2.1.1) n=1 Tax=Homo sapiens RepID=B4DE31_HUMAN B4DE31 (+3)
8. cDNA FLJ59339, highly similar to Probable ATP-dependent RNA helicase DDX5 (EC 3.6.1.-) n=1 Tax=Homo sapiens RepID=B4DLW8_HUMAN B4DLW8 (+1)
9. T-complex protein 1 subunit theta n=2 Tax=Hominidae RepID=TCPQ_HUMAN P50990 (+2)
10. Heterogeneous nuclear ribonucleoprotein K n=4 Tax=Eutheria RepID=HNRPK_HUMAN P61978 (+2)
11. cDNA, FLJ94440, highly similar to Homo sapiens chaperonin containing TCP1, subunit 6A (zeta 1)(CCT6A), mRNA n=1 Tax=Homo sapiens RepID=B2R9K8_HUMAN B2R9K8 (+2)
12. cDNA FLJ53116, highly similar to T-complex protein 1 subunit epsilon n=1 Tax=Homo sapiens RepID=B4DZT5_HUMAN B4DZT5 (+2)
13. Tyrosyl-tRNA synthetase, cytoplasmic n=2 Tax=Homo sapiens RepID=SYYC_HUMAN P54577
14. cDNA FLJ76817, highly similar to Homo sapiens non-POU domain containing, octamer-binding (NONO), mRNA n=1 Tax=Homo sapiens RepID=A8K525_HUMAN A8K525 (+2)
15. Ribosomal L1 domain-containing protein 1 n=3 Tax=Hominidae RepID=RL1D1_HUMAN O76021 (+2)
16. Serum albumin n=1 Tax=Bos taurus RepID=ALBU_BOVIN P02769
17. **Transcriptional repressor protein YY1 n=1 Tax=Homo sapiens RepID=TYY1_HUMAN P25490**
18. ATP-binding cassette sub-family E member 1 n=4 Tax=Euarchontoglires RepID=ABCE1_HUMAN P61221
19. Insulin-like growth factor 2 mRNA-binding protein 1 n=1 Tax=Homo sapiens RepID=IF2B1_HUMAN Q9NZI8
20. Ubiquitin carboxyl-terminal hydrolase n=1 Tax=Homo sapiens RepID=A6NJA2_HUMAN A6NJA2 (+3)
21. cDNA FLJ75056, highly similar to Homo sapiens phosphatidylinositol binding clathrin assembly protein (PICALM), mRNA n=1 Tax=Homo sapiens RepID=A8K5U9_HUMAN A8K5U9 (+8)

22. cDNA FLJ75460, highly similar to Homo sapiens phenylalanine-tRNA synthetase-like, beta subunit, mRNA n=1 Tax=Homo sapiens
RepID=A8K666_HUMANA8K666 (+3)
23. cDNA FLJ51387, highly similar to Asparagine synthetase (glutamine-hydrolyzing) (EC 6.3.5.4) n=1 Tax=Homo sapiens
RepID=B4DY32_HUMAN B4DY32 (+1)
24. Chaperonin containing TCP1, subunit 7 (Eta), isoform CRA_c n=1 Tax=Homo sapiens RepID=B7Z4T9_HUMAN B7Z4T9 (+4)
25. AAA domain containing 3A protein n=2 Tax=Homo sapiens RepID=D2K8Q1_HUMAN D2K8Q1
26. Guanine nucleotide-binding protein-like 3 n=2 Tax=Homo sapiens RepID=GNL3_HUMAN Q9BVP2
27. Nucleolar protein 58 n=1 Tax=Homo sapiens RepID=NOP58_HUMAN Q9Y2X3
28. Putative uncharacterized protein EIF3D n=1 Tax=Homo sapiens RepID=A8MWD3_HUMAN A8MWD3 (+3)
29. cDNA FLJ31567 fis, clone NT2RI2001540, highly similar to Eukaryotic translation initiation factor 3 subunit 6-interacting protein n=1
Tax=Homo sapiens RepID=B3KPB9_HUMAN B3KPB9 (+3)
30. Pyruvate kinase n=1 Tax=Homo sapiens RepID=B4DPM0_HUMAN B4DPM0 (+6)
31. Heat shock cognate 71 kDa protein n=8 Tax=Eutheria RepID=HSP7C_HUMAN P11142