

# 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, GM12878 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 1.8% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

1. ATP synthase subunit beta, mitochondrial n=1 Tax=Homo sapiens RepID=ATPB\_HUMAN P06576 (+1)
2. cDNA FLJ52842, highly similar to Actin, cytoplasmic 1 n=1 Tax=Homo sapiens RepID=B4E335\_HUMAN B4E335 (+7)
3. Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA\_HUMAN P06733
4. Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C\_HUMAN P68371 (+1)
5. Pyruvate kinase isozymes M1/M2 n=2 Tax=Homininae RepID=KPYM\_HUMAN P14618
6. **Interferon regulatory factor 4 n=1 Tax=Homo sapiens RepID=IRF4\_HUMAN Q15306 (+1)**
7. cDNA FLJ78244, highly similar to Homo sapiens eukaryotic translation initiation factor 4A, isoform 1 (EIF4A1), mRNA n=1 Tax=Homo sapiens RepID=A8K7F6\_HUMAN A8K7F6 (+1)
8. ATP synthase subunit alpha, mitochondrial n=3 Tax=Homininae RepID=ATPA\_HUMAN P25705
9. 6-phosphogluconate dehydrogenase, decarboxylating n=3 Tax=Homo sapiens RepID=6PGD\_HUMAN P52209
10. cDNA FLJ78120, highly similar to Homo sapiens eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa (EIF2S3), mRNA n=1 Tax=Homo sapiens RepID=A8K2Y2\_HUMAN A8K2Y2 (+2)
11. cDNA FLJ75299, highly similar to Xenopus laevis proteasome (prosome, macropain) 26S subunit, ATPase 3, mRNA n=1 Tax=Homo sapiens RepID=A8K781\_HUMAN A8K781 (+1)

12. RuvB-like 2 (E. coli), isoform CRA\_d n=1 Tax=Homo sapiens RepID=B3KNL2\_HUMAN  
B3KNL2 (+2)
13. 60S ribosomal protein L4 n=1 Tax=Homo sapiens RepID=RL4\_HUMAN P36578 (+2)
14. 60S ribosomal protein L3 n=1 Tax=Homo sapiens RepID=RL3\_HUMAN P39023 (+4)
15. Tubulin alpha-1C chain n=2 Tax=Homininae RepID=TBA1C\_HUMAN Q9BQE3
16. cDNA FLJ78579, highly similar to Homo sapiens c-src tyrosine kinase (CSK), mRNA n=1  
Tax=Homo sapiens RepID=A8K3B6\_HUMAN A8K3B6 (+4)
17. cDNA FLJ75185 n=1 Tax=Homo sapiens RepID=A8K3D0\_HUMAN A8K3D0 (+10)
18. cDNA FLJ16143 fis, clone BRAMY2038516, highly similar to Protein disulfide-isomerase A6 (EC  
5.3.4.1) n=1 Tax=Homo sapiens RepID=B3KY95\_HUMAN B3KY95 (+4)
19. cDNA FLJ52929, highly similar to Dolichyl-diphosphooligosaccharide--proteinglycosyltransferase  
48 kDa subunit (EC2.4.1.119) n=1 Tax=Homo sapiens RepID=B4DJE3\_HUMAN B4DJE3  
(+3)
20. cDNA FLJ60299, highly similar to Rab GDP dissociation inhibitor beta n=1 Tax=Homo sapiens  
RepID=B4DLV7\_HUMAN B4DLV7 (+3)
21. cDNA FLJ54023, highly similar to Heat shock protein HSP 90-beta n=1 Tax=Homo sapiens  
RepID=B4DMA2\_HUMAN B4DMA2 (+1)
22. cDNA FLJ56389, highly similar to Elongation factor 1-gamma n=1 Tax=Homo sapiens  
RepID=B4DTG2\_HUMAN B4DTG2 (+1)
23. D-3-phosphoglycerate dehydrogenase n=1 Tax=Homo sapiens RepID=SERA\_HUMAN  
O43175
24. Tubulin beta chain n=12 Tax=Amniota RepID=TBB5\_HUMAN P07437
25. Elongation factor Tu, mitochondrial n=1 Tax=Homo sapiens RepID=EFTU\_HUMAN P49411
26. Actin-related protein 3 n=4 Tax=Eutheria RepID=ARP3\_HUMAN P61158
27. Tubulin alpha-4A chain n=8 Tax=Eutheria RepID=TBA4A\_HUMAN P68366
28. Putative uncharacterized protein (Fragment) n=1 Tax=Homo sapiens RepID=Q59G88\_HUMAN  
Q59G88 (+1)
29. keratin, type II cytoskeletal 7 n=1 Tax=Homo sapiens RepID=UPI000013CF9E  
UPI000013CF9E (+1)