

# 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. T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4  
TCPG\_HUMAN
2. Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 K2C1\_HUMAN
3. Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM2 PE=1 SV=4KPYM\_HUMAN
4. Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1  
DDX5\_HUMAN
5. Insulin-like growth factor 2 mRNA-binding protein 1 OS=Homo sapiens GN=IGF2BP1 PE=1  
SV=2 IF2B1\_HUMAN
6. Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6 K1C10\_HUMAN
7. Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK PE=1 SV=1  
HNRPK\_HUMAN
8. Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1  
HSP7C\_HUMAN
9. 60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2  
CH60\_HUMAN
10. Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4  
HS90B\_HUMAN
11. 26S proteasome non-ATPase regulatory subunit 3 OS=Homo sapiens GN=PSMD3 PE=1 SV=2  
PSMD3\_HUMAN
12. Bifunctional purine biosynthesis protein PURH OS=Homo sapiens GN=ATIC PE=1 SV=3  
PUR9\_HUMAN
13. Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3 K1C9\_HUMAN

14. T-complex protein 1 subunit epsilon OS=Homo sapiens GN=CCT5 PE=1 SV=1  
TCPE\_HUMAN
15. Eukaryotic translation initiation factor 3 subunit L OS=Homo sapiens GN=EIF3L PE=1 SV=1  
EIF3L\_HUMAN
16. Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2  
K22E\_HUMAN
17. Cystathionine beta-synthase OS=Homo sapiens GN=CBS PE=1 SV=2 CBS\_HUMAN
18. Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3 TKT\_HUMAN
19. ATP-binding cassette sub-family E member 1 OS=Homo sapiens GN=ABCE1 PE=1 SV=1  
ABCE1\_HUMAN
20. Protein arginine N-methyltransferase 5 OS=Homo sapiens GN=PRMT5 PE=1 SV=4  
ANM5\_HUMAN
21. ATPase family AAA domain-containing protein 3A OS=Homo sapiens GN=ATAD3A PE=1 SV=2  
ATD3A\_HUMAN
22. Insulin-like growth factor 2 mRNA-binding protein 3 OS=Homo sapiens GN=IGF2BP3 PE=1  
SV=2 IF2B3\_HUMAN
23. Phosphoenolpyruvate carboxykinase [GTP], mitochondrial OS=Homo sapiens GN=PCK2 PE=1  
SV=3 PCKGM\_HUMAN
24. Phosphatidylinositol-binding clathrin assembly protein OS=Homo sapiens GN=PICALM PE=1  
SV=2 PICAL\_HUMAN
25. Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Homo sapiens  
GN=RPN1 PE=1 SV=1 RPN1\_HUMAN
- 26. Serum response factor OS=Homo sapiens GN=SRF PE=1 SV=1 SRF\_HUMAN**
27. Phenylalanyl-tRNA synthetase beta chain OS=Homo sapiens GN=FARSB PE=1 SV=3  
SYFB\_HUMAN
28. Arginyl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=RARS PE=1 SV=2  
SYRC\_HUMAN