

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

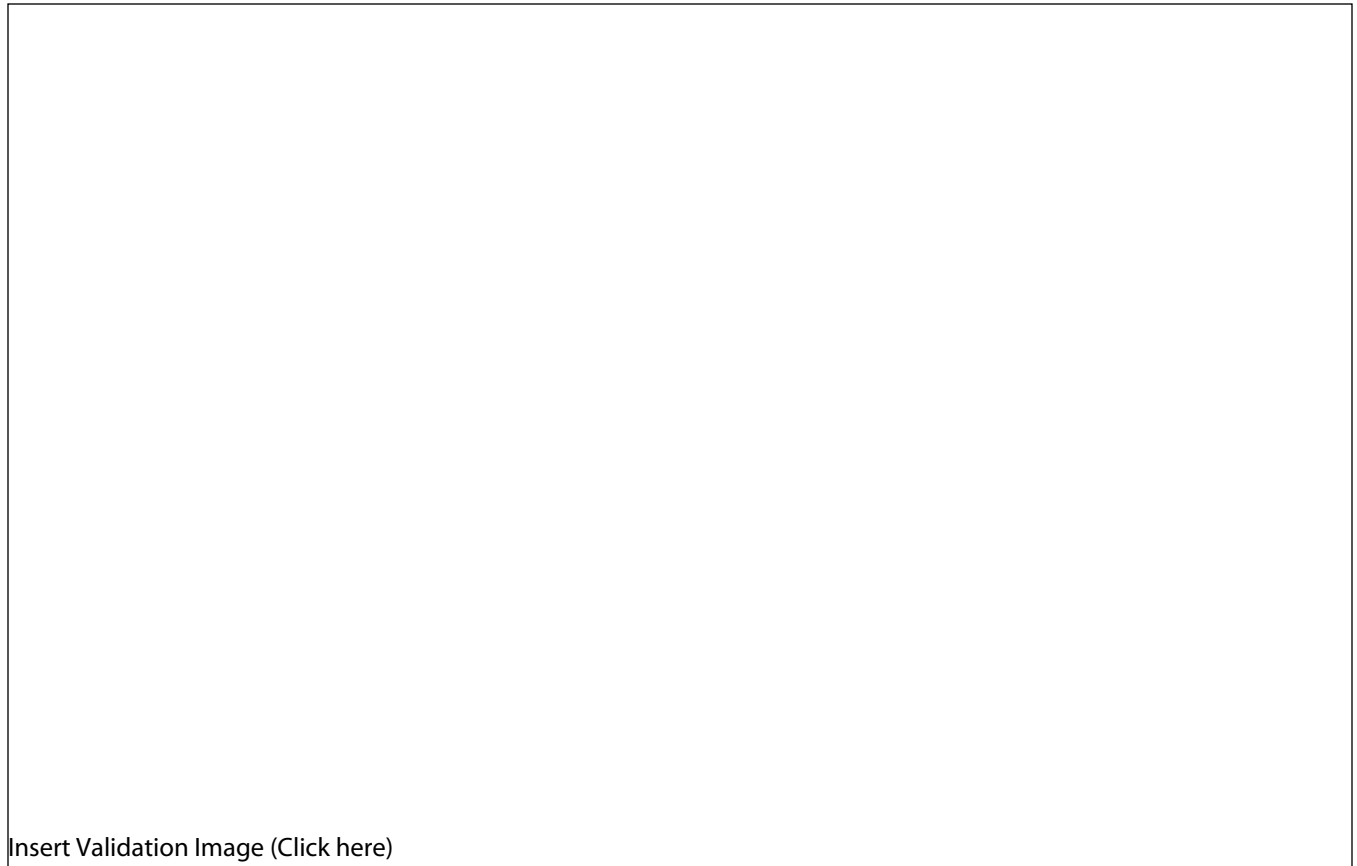
Validation #1
Analysis



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. Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=5
SYEP_HUMAN
2. Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens GN=LRPPRC PE=1
SV=3 LPPRC_HUMAN
3. ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4 DHX9_HUMAN
4. Splicing factor 3B subunit 1 OS=Homo sapiens GN=SF3B1 PE=1 SV=3 SF3B1_HUMAN
5. Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 FAS_HUMAN
6. Isoleucyl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=IARS PE=1 SV=2
SYIC_HUMAN
7. Valyl-tRNA synthetase OS=Homo sapiens GN=VARs PE=1 SV=4 SYVC_HUMAN
8. Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5 CLH1_HUMAN
9. DNA-dependent protein kinase catalytic subunit OS=Homo sapiens GN=PRKDC PE=1 SV=3
PRKDC_HUMAN
10. Leucyl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=LARS PE=1 SV=2
SYLC_HUMAN
11. Hypoxia up-regulated protein 1 OS=Homo sapiens GN=HYOU1 PE=1 SV=1 HYOU1_HUMAN
12. Exportin-5 OS=Homo sapiens GN=XPO5 PE=1 SV=1 XPO5_HUMAN
- 13. Paired amphipathic helix protein Sin3a OS=Homo sapiens GN=SIN3A PE=1 SV=2
SIN3A_HUMAN**
14. Coatamer subunit alpha OS=Homo sapiens GN=COPA PE=1 SV=2 COPA_HUMAN
15. Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 K2C1_HUMAN
16. Protein diaphanous homolog 1 OS=Homo sapiens GN=DIAPH1 PE=1 SV=2 DIAP1_HUMAN

17. Eukaryotic translation initiation factor 3 subunit A OS=Homo sapiens GN=EIF3A PE=1 SV=1
EIF3A_HUMAN
18. Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3 K1C9_HUMAN
19. Myb-binding protein 1A OS=Homo sapiens GN=MYBBP1A PE=1 SV=2 MBB1A_HUMAN
20. Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4 MYH9_HUMAN
21. Ras GTPase-activating-like protein IQGAP1 OS=Homo sapiens GN=IQGAP1 PE=1 SV=1
IQGA1_HUMAN
22. Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16 OS=Homo sapiens GN=DHX38
PE=1 SV=2 PRP16_HUMAN