ENCODE DCC Antibody Validation Document

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Name: Florencia Pauli

Email: fpauli@hudsonalpha.org

Lab: Myers

Antibody Name: PAX5-C20

Target: PAX5

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-1794

Lot Number: E1409

Antibody Description: Goat polyclonal IgG. Epitope mapping at the C-terminus of Pax-5 of human origin.

Target Description: PAX proteins are important regulators in early development, and alterations in the expression of their genes are thought to contribute to neoplastic transformation.

Entrez Gene ID 5097. 46 kDa

Species Target: Human

Species Host: Goat

Validation Method #1: Western Blot

Validation Method #2: IP-Mass Spec

Purification Method:

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://www.scbt.com/datasheet-1974-pax-5-

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

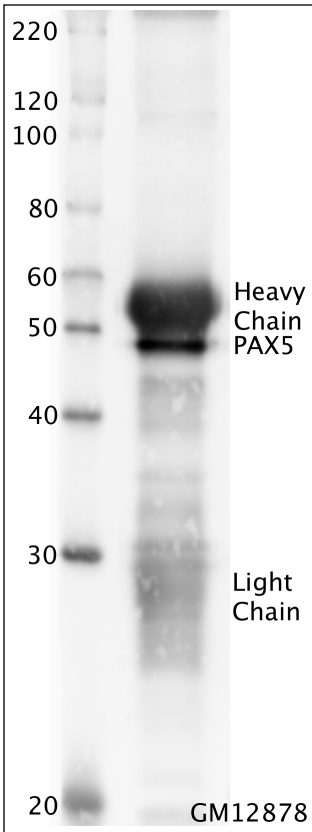
Histone Name | AA modified | AA Position | Modification

Validation #1
Analysis

Western blot protocol:
Whole cell lysate was 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. After separation, the samples were transferred to a nitrocellulose membrane with an Invitrogen iBlot system. Blotting with primary (same as that used for IP) and secondary HRP-conjugated antibodies was performed on an Invitrogen BenchPro 4100 system. Visualization was achieved using SuperSignal West Femto solution (Thermo Scientific).

Results: Band of expected size (~46 kDa) visualized, representing strongest signal in the lane.

Figure legend: IP-western with sc-1974 in whole cell lysate (WCL) of GM12878; PM=protein marker. PAX5 band is indicated.



Insert Validation Image (click here)

Validation #2 Analysis	IP followed by mass spectrometry: 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. Gel fragments corresponding to the bands indicated above in the western blot image were excised and sent to the University of Alabama at Birmingham Cancer Center Mass Spectrometry/Proteomics Shared Facility. There the samples were run on an LTQ XL Linear Ion Trap Mass Spectrometer by LC-ESI-MS/MS. Peptides were identified using SEQUEST tandem mass spectra analysis, with probability based matching at $p < 0.05$. As per ENCODE data standards, all SEQUEST results are attached (ENCODE_HAIB_PAX5_sc1974_09122011_MassSpec.pdf), including common contaminants. Target protein is listed as hit 19 in ~46 kDa band.

Insert Validation Image (Click here)

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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, 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 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 $p < 0.05$. 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. Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 ACTB_HUMAN (+1)
2. Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1 EF1A1_HUMAN
3. Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 ALBU_HUMAN
4. Phosphoglycerate kinase 1 OS=Homo sapiens GN=PGK1 PE=1 SV=3 PGK1_HUMAN
5. Fibrinogen alpha chain OS=Homo sapiens GN=FGA PE=1 SV=2 FIBA_HUMAN
6. Fibrinogen beta chain OS=Homo sapiens GN=FGB PE=1 SV=2 FIBB_HUMAN
7. Fibrinogen gamma chain OS=Homo sapiens GN=FGG PE=1 SV=3 FIBG_HUMAN
8. Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1 HPT_HUMAN
9. 3-ketoacyl-CoA thiolase, mitochondrial OS=Homo sapiens GN=ACAA2 PE=1 SV=2 THIM_HUMAN
10. Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3 A1AT_HUMAN
11. Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1 APOA1_HUMAN
12. Actin-related protein 2 OS=Homo sapiens GN=ACTR2 PE=1 SV=1 ARP2_HUMAN
13. Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2 CO3_HUMAN
14. Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 ENOA_HUMAN
15. Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens GN=IDH2 PE=1 SV=2 IDHP_HUMAN
16. Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2 IGHA1_HUMAN (+1)
17. Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1 IGKC_HUMAN
18. Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM2 PE=1 SV=4KPYM_HUMAN
19. **Paired box protein Pax-5 OS=Homo sapiens GN=PAX5 PE=1 SV=1 PAX5_HUMAN**

20. Cytochrome b-c1 complex subunit 2, mitochondrial OS=Homo sapiens GN=UQCRC2 PE=1 SV=3 QCR2_HUMAN
21. Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=2 TRFE_HUMAN