ENCODE Antibody Validation Documentation
Transcription factor: Paired box 5 (GeneID 5079)

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Transcription factor: PAX5 (GeneID 5079; ~42 kDa)

Antibody: PAX-5 (C-20), Santa Cruz Biotechnology (sc-1974)
Goat polyclonal, epitope mapping at the C-terminus of PAX-5 of human origin
Web: http://www.scbt.com/datasheet-1974-pax-5-c-20-antibody.html

Validation 1: Immunoblot Analysis

For an antibody to meet ENCODE validation standards, a single band of the predicted size, or a band of no less than half the total signal, must be detected in a lane on a Western blot.

a. Vendor immunoblot analysis

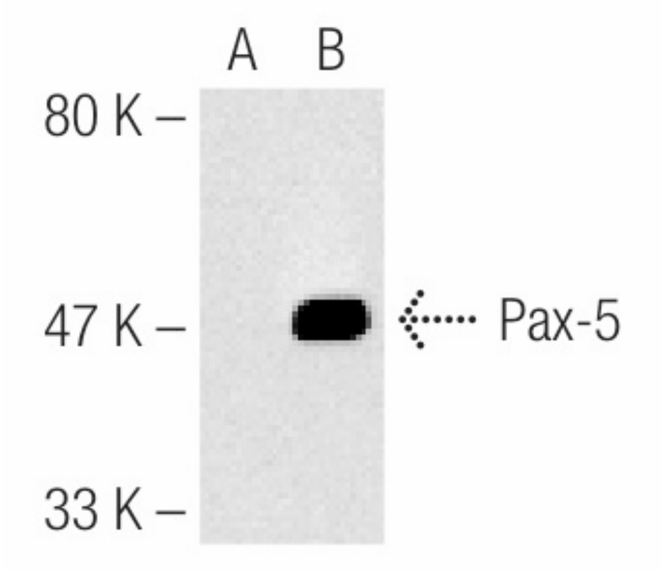

Figure Legend: Western blot analysis of PAX-5 expression in WEHI-231 (A) and U-937 (B) whole cell lysates.

b. Myers Lab immunoblot analysis

Western blot protocol

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. After separation, the samples were transferred to a nitrocellulose membrane using a Bio-Rad Trans-Blot Electrophoretic Transfer system. Standard western blot protocol was used to probe the membrane with the primary antibody (same antibody as used for IP), and an HRP-conjugated secondary antibody and SuperSignal West Femto solution (Thermo Scientific) were used to detect the immunoprecipitated proteins.

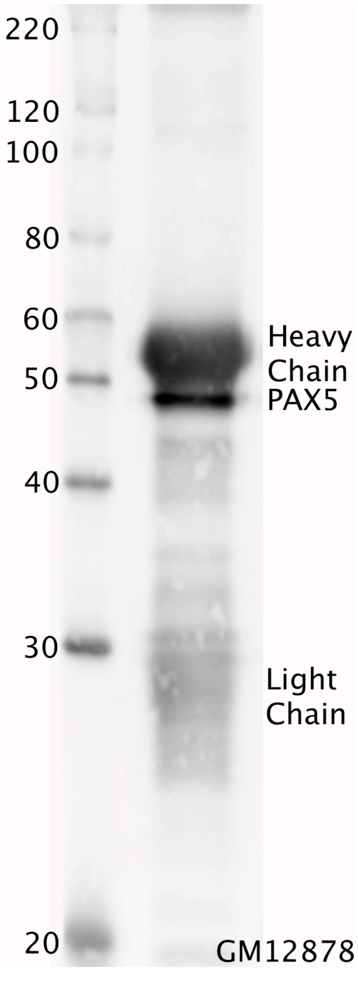

Figure Legend: PAX5 immunoblot: IP-western with sc-1974 PAX-5 antibody in whole cell lysate of GM12878. Heavy and light chains of IgG are indicated, and PAX5 band is indicated at ~47 kDa.

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.

Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 ACTB_HUMAN (+1)

Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1 EF1A1_HUMAN

Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 ALBU_HUMAN

Phosphoglycerate kinase 1 OS=Homo sapiens GN=PGK1 PE=1 SV=3 PGK1_HUMAN

Fibrinogen alpha chain OS=Homo sapiens GN=FGA PE=1 SV=2 FIBA_HUMAN

Fibrinogen beta chain OS=Homo sapiens GN=FGB PE=1 SV=2 FIBB_HUMAN

Fibrinogen gamma chain OS=Homo sapiens GN=FGG PE=1 SV=3 FIBG_HUMAN

Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1 HPT_HUMAN

3-ketoacyl-CoA thiolase, mitochondrial OS=Homo sapiens GN=ACAA2 PE=1 SV=2 THIM_HUMAN

Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3 A1AT_HUMAN

Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1 APOA1_HUMAN

Actin-related protein 2 OS=Homo sapiens GN=ACTR2 PE=1 SV=1 ARP2_HUMAN

Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2 CO3_HUMAN

Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 ENOA_HUMAN

Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens GN=IDH2 PE=1 SV=2 IDHP_HUMAN

Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2 IGHA1_HUMAN (+1)

Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1 IGKC_HUMAN

Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM2 PE=1 SV=4 KPYM_HUMAN

Paired box protein Pax-5 OS=Homo sapiens GN=PAX5 PE=1 SV=1 PAX5_HUMAN

Cytochrome b-c1 complex subunit 2, mitochondrial OS=Homo sapiens GN=UQCRC2 PE=1 SV=3 QCR2_HUMAN

Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=2 TRFE_HUMAN