ENCODE Antibody Validation Documentation
Transcription factor: E2F transcription factor 6 (GeneID 1876)

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Transcription factor: E2F6 (GeneID 1876; ~32 kDa)

Antibody: E2F6 (H-50), Santa Cruz Biotechnology (sc-22823)
Rabbit polyclonal, epitope corresponding to amino acids 232-281 mapping at the C-terminus of E2F6 of human origin
Web: http://www.scbt.com/datasheet-22823-e2f-6-h-50-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 E2F6 expression in K562 nuclear extract.
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 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).

Figure Legend: E2F6 immunoblot: IP-western with sc-22823 E2F6 antibody in whole cell lysates (WCL) of K562 and HepG2. Heavy chain and light chain of IgG are indicated, and E2F6 band is indicated at ~38 kDa.
Validation 2: Mass Spectrometry Analysis

ENCODE data standards recognize 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 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. As per ENCODE data standards, all MASCOT results are listed below, including common contaminants. Target protein is highlighted in bold font.

P07195  L-lactate dehydrogenase B chain n=4 Tax=Catarrhini RepID=LDHB_HUMAN

P04264  Keratin, type II cytoskeletal 1 n=1 Tax=Homo sapiens RepID=K2C1_HUMAN

A8K4Z4  cDNA FLJ75549, highly similar to Homo sapiens ribosomal protein, large, P0 (RPLP0), transcript variant 1, mRNA n=1 Tax=Homo sapiens RepID=A8K4Z4_HUMAN

P07900  Heat shock protein HSP 90-alpha n=2 Tax=Homo sapiens RepID=HS90A_HUMAN

Q53F48  Heterogeneous nuclear ribonucleoprotein H3 isoform a variant (Fragment) n=1 Tax=Homo sapiens RepID=Q53F48_HUMAN

UPI00017BCE7F keratin, type I cytoskeletal 10 n=1 Tax=Homo sapiens RepID=UPI00017BCE7F

P35908  Keratin, type II cytoskeletal 2 epidermal n=1 Tax=Homo sapiens RepID=K22E_HUMAN

B2R5W2  cDNA, FLJ92657, highly similar to Homo sapiens heterogeneous nuclear ribonucleoprotein C (C1/C2) (HNRPC), transcript variant 2, mRNA n=1 Tax=Homo sapiens RepID=B2R5W2_HUMAN

Q9BQE3  Tubulin alpha-1C chain n=2 Tax=Homininae RepID=TBA1C_HUMAN

Q15365  Poly(rC)-binding protein 1 n=7 Tax=Eutheria RepID=PCBP1_HUMAN

UPI0001AE6927  UPI0001AE6927 related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE6927

P29692  Elongation factor 1-delta n=2 Tax=Homo sapiens RepID=EF1D_HUMAN

C9J4M5  L-lactate dehydrogenase n=1 Tax=Homo sapiens RepID=C9J4M5_HUMAN

B3KNJ4  SUMO-1 activating enzyme subunit 1, isoform CRA_a n=1 Tax=Homo sapiens RepID=B3KNJ4_HUMAN

B3KMQ2  cDNA FLJ11916 fis, clone HEMBB1000217, highly similar to Ubiquitin-like 1-activating enzyme E1A n=1 Tax=Homo sapiens RepID=B3KMQ2_HUMAN

P04075  Fructose-bisphosphate aldolase A n=1 Tax=Homo sapiens RepID=ALDOA_HUMAN

P08238  Heat shock protein HSP 90-beta n=1 Tax=Homo sapiens RepID=HS90B_HUMAN
P06733  Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA_HUMAN
B4DXP5  cDNA FLJ58339, highly similar to Poly(rC)-binding protein 2 n=2 Tax=Euarchontoglires RepID=B4DXP5_HUMAN
UPI000186DDE4 tubulin alpha-3 chain, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186DDE4
P05198  Eukaryotic translation initiation factor 2 subunit 1 n=2 Tax=Homo sapiens RepID=IF2A_HUMAN
A8MV37  Putative uncharacterized protein SEC13 n=1 Tax=Homo sapiens RepID=A8MV37_HUMAN
P35527  Keratin, type I cytoskeletal 9 n=1 Tax=Homo sapiens RepID=K1C9_HUMAN
Q3ZCQ8  Mitochondrial import inner membrane translocase subunit TIM50 n=1 Tax=Homo sapiens RepID=TIM50_HUMAN
B2RBR9  cDNA, FLJ95650, highly similar to Homo sapiens karyopherin (importin) beta 1 (KPNB1), mRNA n=1 Tax=Homo sapiens RepID=B2RBR9_HUMAN
Q5ST81  Tubulin beta polypeptide n=3 Tax=Hominidae RepID=Q5ST81_HUMAN
B3KTV0  cDNA FLJ38781 fis, clone LIVER2000216, highly similar to HEAT SHOCK COGNATE 71 kDa PROTEIN n=1 Tax=Homo sapiens RepID=B3KTV0_HUMAN
P31942-5 Isoform 5 of Heterogeneous nuclear ribonucleoprotein H3 n=1 Tax=Homo sapiens RepID=P31942-5
P46109  Crk-like protein n=2 Tax=Homo sapiens RepID=CRKL_HUMAN
B4DLT1  cDNA FLJ59716, highly similar to Vacuolar protein sorting 26A n=1 Tax=Homo sapiens RepID=B4DLT1_HUMAN
Q0IIN1  Keratin 77 n=1 Tax=Homo sapiens RepID=Q0IIN1_HUMAN
Q9BTI9  NPM1 protein (Fragment) n=2 Tax=Homininae RepID=Q9BTI9_HUMAN
UPI000186D28A ribosomal P0 protein, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186D28A
Q7L2H7  Eukaryotic translation initiation factor 3 subunit M n=1 Tax=Homo sapiens RepID=EIF3M_HUMAN
P04406  Glyceraldehyde-3-phosphate dehydrogenase n=1 Tax=Homo sapiens RepID=G3P_HUMAN
Q13347  Eukaryotic translation initiation factor 3 subunit I n=2 Tax=Homo sapiens RepID=EIF3I_HUMAN
A4ZU86  Truncated nucleolar phosphoprotein B23 n=1 Tax=Homo sapiens RepID=A4ZU86_HUMAN
Q9BTI9  NPM1 protein (Fragment) n=2 Tax=Homininae RepID=Q9BTI9_HUMAN
UPI000179EC85 Serum albumin precursor (Allergen Bos d 6) (BSA). n=1 Tax=Bos taurus RepID=UPI000179EC85
B7Z3I7  Malate dehydrogenase n=1 Tax=Homo sapiens RepID=B7Z3I7_HUMAN
B4DDU2  cDNA FLJ60097, highly similar to Tubulin alpha-ubiquitous chain n=1 Tax=Homo sapiens RepID=B4DDU2_HUMAN

P17987  T-complex protein 1 subunit alpha n=2 Tax=Homininae RepID=TCPA_HUMAN

P53004  Biliverdin reductase A n=1 Tax=Homo sapiens RepID=BIEA_HUMAN

UPI0001AE669E  UPI0001AE669E related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE669E

Q6Q9Z6  E2F6 splice variant b n=1 Tax=Homo sapiens RepID=Q6Q9Z6_HUMAN

D3DP77  Heterogeneous nuclear ribonucleoprotein A/B, isoform CRA_b n=2 Tax=Homo sapiens RepID=D3DP77_HUMAN

UPI0001AE6729  UPI0001AE6729 related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE6729

A8MWR8  Putative uncharacterized protein SEC13 n=1 Tax=Homo sapiens RepID=A8MWR8_HUMAN

UPI0001AE634C  Probable ATP-dependent RNA helicase DDX17 (EC 3.6.1.-) (DEAD box protein 17) (RNA-dependent helicase p72) (DEAD box protein p72). n=1 Tax=Homo sapiens RepID=UPI0001AE634C

Q14979  Heterogeneous nuclear ribonucleoprotein D-like n=1 Tax=Homo sapiens RepID=HNRDL_HUMAN

B9ZVU1  Putative uncharacterized protein HNRNPD n=1 Tax=Homo sapiens RepID=B9ZVU1_HUMAN

UPI0000F2B487  PREDICTED: similar to nucleophosmin - human n=1 Tax=Monodelphis domestica RepID=UPI0000F2B487

P36873  Serine/threonine-protein phosphatase PP1-gamma catalytic subunit n=2 Tax=Catarrhini RepID=PP1G_HUMAN

B4E163  Serine/threonine-protein phosphatase n=2 Tax=Homo sapiens RepID=B4E163_HUMAN

B7ZB67  Serine/threonine-protein phosphatase n=1 Tax=Homo sapiens RepID=B7ZB67_HUMAN

B4DJ75  Serine/threonine-protein phosphatase n=1 Tax=Homo sapiens RepID=B4DJ75_HUMAN

Q6IBN4  PECI protein n=1 Tax=Homo sapiens RepID=Q6IBN4_HUMAN

Q9Y3A4  Ribosomal RNA-processing protein 7 homolog A n=1 Tax=Homo sapiens RepID=RRP7A_HUMAN

Q9BYG3  MKI67 FHA domain-interacting nucleolar phosphoprotein n=1 Tax=Homo sapiens RepID=MK67I_HUMAN

B7Z2X9  Enolase n=1 Tax=Homo sapiens RepID=B7Z2X9_HUMAN

Q58FF8  Putative heat shock protein HSP 90-beta 2 n=1 Tax=Homo sapiens RepID=H90B2_HUMAN

B5MCE8  Putative uncharacterized protein BUB3 n=1 Tax=Homo sapiens RepID=B5MCE8_HUMAN

UPI0001AE68FD  UPI0001AE68FD related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE68FD

B4DMJ6  cDNA FLJ50996, highly similar to 60S ribosomal protein L4 n=1 Tax=Homo sapiens RepID=B4DMJ6_HUMAN
O75367  Core histone macro-H2A.1 n=2 Tax=Eutheria RepID=H2AY_HUMAN
Q9NYD2  Hepatocellular carcinoma-associated antigen 64 n=1 Tax=Homo sapiens RepID=Q9NYD2_HUMAN
B8ZZ37  Putative uncharacterized protein HNRNPA2B1 n=1 Tax=Homo sapiens RepID=B8ZZ37_HUMAN
A6NN60  Putative uncharacterized protein CRYZ n=1 Tax=Homo sapiens RepID=A6NN60_HUMAN
C5IWV5  Trypsinogen n=1 Tax=Sus scrofa RepID=C5IWV5_PIG
P17516  Aldo-keto reductase family 1 member C4 n=1 Tax=Homo sapiens RepID=AK1C4_HUMAN
B4DK69  cDNA FLJ52680, highly similar to Aldo-keto reductase family 1 member C2 (EC 1.1.1.1) n=1 Tax=Homo sapiens RepID=B4DK69_HUMAN
A8K0T9  cDNA FLJ75422, highly similar to Homo sapiens capping protein (actin filament) muscle Z-line, alpha 1, mRNA n=1 Tax=Homo sapiens RepID=A8K0T9_HUMAN
B7ZW38  LOC649330 protein n=1 Tax=Homo sapiens RepID=B7ZW38_HUMAN
A8K854  HCG1983504, isoform CRA_f n=1 Tax=Homo sapiens RepID=A8K854_HUMAN
Q7KZS6  HCG2042771 n=1 Tax=Homo sapiens RepID=Q7KZS6_HUMAN
Q32P51  Heterogeneous nuclear ribonucleoprotein A1-like 2 n=1 Tax=Homo sapiens RepID=[A1L2_HUMAN]
B5BU08  U2 small nuclear RNA auxiliary factor 1 isoform a n=1 Tax=Homo sapiens RepID=B5BU08_HUMAN
Q9NPL4  Enolase (Fragment) n=1 Tax=Homo sapiens RepID=Q9NPL4_HUMAN
Q04828  Aldo-keto reductase family 1 member C1 n=1 Tax=Homo sapiens RepID=AK1C1_HUMAN
Q2XPP3  Type II 3a-hydroxysteroid dehydrogenase variant n=1 Tax=Homo sapiens RepID=Q2XPP3_Human
Q9NR45  Sialic acid synthase n=1 Tax=Homo sapiens RepID=SIAS_HUMAN
B5BU02  Replication factor C 2 isoform 1 (Fragment) n=1 Tax=Homo sapiens RepID=B5BU02_HUMAN
Q5FWY2  GNAS complex locus n=1 Tax=Homo sapiens RepID=Q5FWY2_HUMAN
UPI0001866D22  GTP-binding protein alpha subunit, gna, putative n=1 Tax=Pediculus humanus corporis RepID=UPI0001866D22
P63096  Guanine nucleotide-binding protein G(i) subunit alpha-1 n=4 Tax=Eutheria RepID=GNAI1_HUMAN
Q14344  Guanine nucleotide-binding protein subunit alpha-13 n=1 Tax=Homo sapiens RepID=GNA13_HUMAN
P38405  Guanine nucleotide-binding protein G(olf) subunit alpha n=2 Tax=Homo sapiens RepID=GNAL_HUMAN
UPI00015E05E4  MAD2L1-binding protein (Caught by MAD2 protein). n=1 Tax=Homo sapiens RepID=UPI00015E05E4
Q05C15  DHX9 protein (Fragment) n=1 Tax=Homo sapiens RepID=Q05C15_HUMAN
UPI000186CFD7  tubulin beta chain, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186CFD7
Q59EJ3  Heat shock 70kDa protein 1A variant (Fragment) n=1 Tax=Homo sapiens RepID=Q59EJ3_HUMAN
B4E3B6  cDNA FLJ54408, highly similar to Heat shock 70 kDa protein 1 n=1 Tax=Homo sapiens RepID=B4E3B6_HUMAN
Q6FGM6  PPID protein n=1 Tax=Homo sapiens RepID=Q6FGM6_HUMAN
Q9BVE2  CDC2L5 protein n=1 Tax=Homo sapiens RepID=Q9BVE2_HUMAN
B3KPS6  cDNA FLJ32137 fis, clone PEBLM2000479, highly similar to Proto-oncogene tyrosine-protein kinase Fyn (EC 2.7.10.2) n=1 Tax=Homo sapiens RepID=B3KPS6_HUMAN
Q00534  Cell division protein kinase 6 n=2 Tax=Homo sapiens RepID=CDK6_HUMAN
Q9NYV4  Cell division protein kinase 12 n=1 Tax=Homo sapiens RepID=CDK12_HUMAN
B2RA70  cDNA, FLJ94729, highly similar to Homo sapiens v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1(YES1), mRNA n=1 Tax=Homo sapiens RepID=B2RA70_HUMAN
UPI000186E29F  tyrosine-protein kinase Src42A, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186E29F
UPI0001AE6E31  UPI0001AE6E31 related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE6E31
UPI0001AE77E0  UPI0001AE77E0 related cluster n=1 Tax=Homo sapiens RepID=UPI0001AE77E0
Q00537  Cell division protein kinase 17 n=2 Tax=Homo sapiens RepID=CDK17_HUMAN
P50750-2  Isoform 2 of Cell division protein kinase 9 n=1 Tax=Homo sapiens RepID=P50750-2
UPI00001A651A  UPI00001A651A related cluster n=1 Tax=Homo sapiens RepID=UPI00001A651A
UPI000186D3DD  mitogen-activated protein kinase ERK-A, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186D3DD
UPI000186EE41  tyrosine kinase receptor, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186EE41
Q6NUK7  LYN protein (Fragment) n=1 Tax=Homo sapiens RepID=Q6NUK7_HUMAN
UPI0001B79070  Tyrosine-protein kinase HCK (EC 2.7.10.2) (p59-HCK/p60-HCK) (Hemopoietic cell kinase). n=1 Tax=Homo sapiens RepID=UPI0001B79070
UPI000186E55E  mitogen-activated protein kinase ERK-A, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186E55E
Q4R378  Testis cDNA clone: QtsA-18883, similar to human male germ cell-associated kinase (MAK), n=1 Tax=Macaca fascicularis RepID=Q4R378_MACFA
UPI000186DC99   mitogen-activated protein kinase ERK-A, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186DC99
Q96QR8     Transcriptional activator protein Pur-beta n=1 Tax=Homo sapiens RepID=PURB_HUMAN
B4DFF1     cDNA FLJ53312, highly similar to Heterogeneous nuclear ribonucleoprotein K n=1 Tax=Homo sapiens RepID=B4DFF1_HUMAN
UPI000186E7BC conserved hypothetical protein n=1 Tax=Pediculus humanus corporis RepID=UPI000186E7BC
Q4G148     Glucoside xylosyltransferase 1 n=1 Tax=Homo sapiens RepID=GXLT1_HUMAN