

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

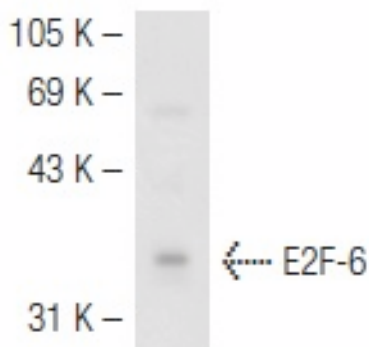


Figure Legend: Western blot analysis of E2F6 expression in K562 nuclear extract.

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).

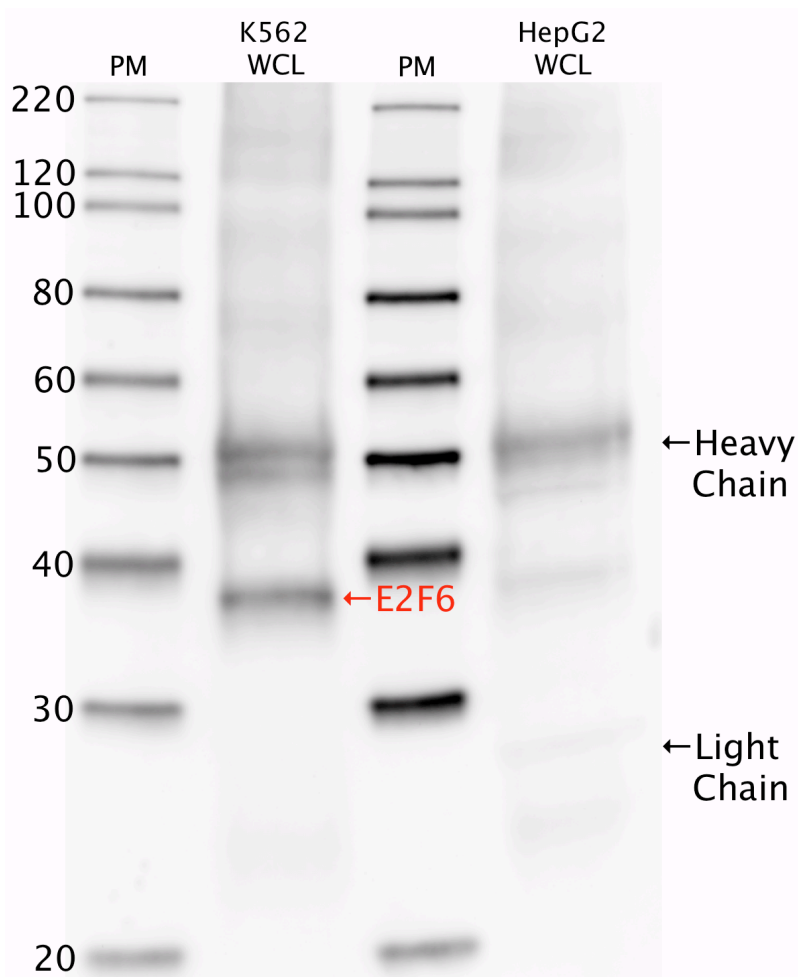


Figure Legend: E2F6 immunoblot: IP-western with sc-22823 E2F6 antibody in whole cell lysates (WCL) of K562 and HepG2. Heavy chain and light chain of IgG are indicated, and E2F6 band is indicated at ~38 kDa.

Validation 2: Mass Spectrometry Analysis

ENCODE data standards 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 $p < 0.05$. 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

P49411 Elongation factor Tu, mitochondrial n=1 Tax=Homo sapiens RepID=EFTU_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

D3DWP7 Heterogeneous nuclear ribonucleoprotein A/B, isoform CRA_b n=2 Tax=Homo sapiens RepID=D3DWP7_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

O14979 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 PECT 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

C5IWW5 Trypsinogen n=1 Tax=Sus scrofa RepID=C5IWW5_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.-.-) 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=RA1L2_HUMAN

B5BU08 U2 small nuclear RNA auxillary 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

B5BUD2 Replication factor C 2 isoform 1 (Fragment) n=1 Tax=Homo sapiens RepID=B5BUD2_HUMAN

Q5FWY2 GNAS complex locus n=1 Tax=Homo sapiens RepID=Q5FWY2_HUMAN

UPI000186D622 GTP-binding protein alpha subunit, gna, putative n=1 Tax=Pediculus humanus corporis RepID=UPI000186D622

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

Q05CI5 DHX9 protein (Fragment) n=1 Tax=Homo sapiens RepID=Q05CI5_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-src-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