ENCODE DCC Antibody Validation Document

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Lab: Myers

Antibody Name: E2F-6 (H-50)

Target: E2F6

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-22823

Lot Number: D2106

Antibody Description: Rabbit polyclonal, epitope corresponding to amino acids 232-281 mapping at the C-terminus of E2F6 of human origin

Target Description: E2F transcription factor 6; ~32 kDa
Entrez Gene: 1876

Species Target: Human

Species Host: Rabbit

Validation Method #1: Western Blot

Validation Method #2: IP-Mass Spec

Purification Method:

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://www.scbt.com/datasheet-22823-

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:

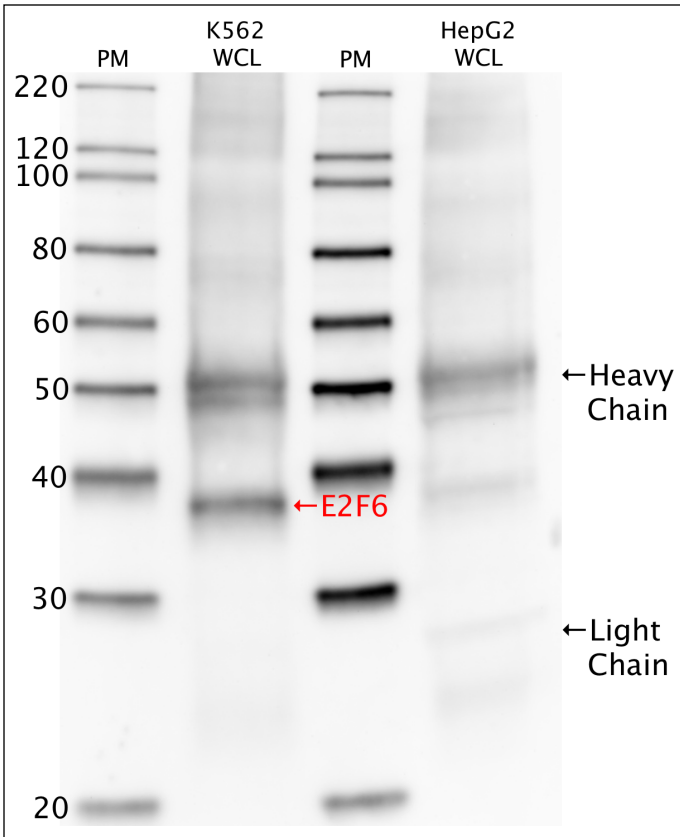
Modification:

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 visualized, representing strongest signal in the lane.

Figure legend: IP-western with sc-22823 in whole cell lysates (WCL) of K562 and HepG2; PM=protein marker. E2F6 band is indicated, as are heavy and light chains of IgG.



Insert Validation Image (click here)

Validation #2 Analysis	IP followed by mass spectrometry: 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 by LC-ESI-MS/MS. Peptides were identified using MASCOT tandem mass spectra analysis, with probability based matching at $p < 0.05$. As per ENCODE data standards, all MASCOT results are attached, including common contaminants. Target protein is listed as hit 41 (row 42 on attached spreadsheet).

Insert Validation Image (Click here)

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Identified Proteins (324)	Accession Number	Molecular Weight	GB E2F6
L-lactate dehydrogenase B chain n=4 Tax=Catarrhini RepID=LDHB_HUMAN	P07195	37 kDa	11
cDNA FLJ75549, highly similar to Homo sapiens ribosomal protein, large, P0 (RPLP0), transcript variant 1, mRNA n=1 Tax=Homo sapiens RepID=A8K4Z4_HUMAN	A8K4Z4 (+1)	34 kDa	9
Poly(rC)-binding protein 1 n=7 Tax=Eutheria RepID=PCBP1_HUMAN	Q15365	37 kDa	8
Fructose-bisphosphate aldolase A n=1 Tax=Homo sapiens RepID=ALDOA_HUMAN	P04075	39 kDa	7
Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA_HUMAN	P06733	47 kDa	5
Eukaryotic translation initiation factor 2 subunit 1 n=2 Tax=Homo sapiens RepID=IF2A_HUMAN	P05198	36 kDa	5
Eukaryotic translation initiation factor 3 subunit I n=2 Tax=Homo sapiens RepID=EIF3I_HUMAN	Q13347	37 kDa	5
Heterogeneous nuclear ribonucleoprotein H3 isoform a variant (Fragment) n=1 Tax=Homo sapiens RepID=Q53F48_HUMAN	Q53F48 (+3)	37 kDa	5
cDNA FLJ54023, highly similar to Heat shock protein HSP 90-beta n=1 Tax=Homo sapiens RepID=B4DMA2_HUMAN	B4DMA2 (+1)	79 kDa	4
cDNA FLJ75154, highly similar to Homo sapiens heterogeneous nuclear ribonucleoprotein C	A8K9A4 (+6)	34 kDa	4

(C1/C2), mRNA n=1 Tax=Homo sapiens RepID=A8K9A4_HUMAN			
cDNA, FLJ95068, highly similar to Homo sapiens eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein) (EEF1D), transcript variant 1, mRNA n=1 Tax=Homo sapiens RepID=B2RAR6_HUMAN	B2RAR6 (+6)	71 kDa	4
Core histone macro-H2A.1 n=2 Tax=Eutheria RepID=H2AY_HUMAN	O75367 (+2)	40 kDa	4
Glyceraldehyde-3-phosphate dehydrogenase n=1 Tax=Homo sapiens RepID=G3P_HUMAN	P04406 (+1)	36 kDa	4
Isoform 2 of L-lactate dehydrogenase A chain n=1 Tax=Homo sapiens RepID=P00338-2	P00338-2 (+2)	36 kDa	4
Serum albumin n=1 Tax=Bos taurus RepID=ALBU_BOVIN	P02769	69 kDa	4
Translocase of inner mitochondrial membrane 50 homolog (S. cerevisiae) n=1 Tax=Homo sapiens RepID=Q0VAB1_HUMAN	Q0VAB1 (+2)	50 kDa	4
Biliverdin reductase A n=1 Tax=Homo sapiens RepID=BIEA_HUMAN	P53004	33 kDa	3
cDNA FLJ59716, highly similar to Vacuolar protein sorting 26A n=1 Tax=Homo sapiens RepID=B4DLT1_HUMAN	B4DLT1 (+1)	37 kDa	3
Eukaryotic translation initiation factor 3 subunit M n=1 Tax=Homo sapiens RepID=EIF3M_HUMAN	Q7L2H7	43 kDa	3

Malate dehydrogenase n=1 Tax=Homo sapiens RepID=B7Z3I7_HUMAN	B7Z3I7 (+3)	39 kDa	3
PECI protein n=1 Tax=Homo sapiens RepID=Q6IBN4_HUMAN	Q6IBN4	40 kDa	3
Putative uncharacterized protein SEC13 n=1 Tax=Homo sapiens RepID=A8MV37_HUMAN	A8MV37 (+1)	34 kDa	3
SUMO-1 activating enzyme subunit 1, isoform CRA_a n=1 Tax=Homo sapiens RepID=B3KNJ4_HUMAN	B3KNJ4 (+4)	33 kDa	3
Tubulin alpha-1C chain n=2 Tax=Hominidae RepID=TBA1C_HUMAN	Q9BQE3	50 kDa	3
60S ribosomal protein L4 n=1 Tax=Homo sapiens RepID=RL4_HUMAN	P36578 (+2)	48 kDa	2
cDNA FLJ58339, highly similar to Poly(rC)-binding protein 2 n=2 Tax=Euarchontoglires RepID=B4DXP5_HUMAN	B4DXP5 (+8)	34 kDa	2
cDNA FLJ59339, highly similar to Probable ATP-dependent RNA helicase DDX5 (EC 3.6.1.-) n=1 Tax=Homo sapiens RepID=B4DLW8_HUMAN	B4DLW8 (+3)	61 kDa	2
cDNA, FLJ95650, highly similar to Homo sapiens karyopherin (importin) beta 1 (KPNB1), mRNA n=1 Tax=Homo sapiens RepID=B2RBR9_HUMAN	B2RBR9 (+1)	97 kDa	2
Crk-like protein n=2 Tax=Homo sapiens RepID=CRKL_HUMAN	P46109	34 kDa	2
Elongation factor 1-alpha 1 n=13 Tax=Eutheria RepID=EF1A1_HUMAN	P68104 (+6)	50 kDa	2

Elongation factor Tu, mitochondrial n=1 Tax=Homo sapiens RepID=EFTU_HUMAN	P49411	50 kDa	2
Heat shock cognate 71 kDa protein n=8 Tax=Eutheria RepID=HSP7C_HUMAN	P11142	71 kDa	2
Heat shock protein HSP 90-alpha n=2 Tax=Homo sapiens RepID=HS90A_HUMAN	P07900	85 kDa	2
MKI67 FHA domain-interacting nucleolar phosphoprotein n=1 Tax=Homo sapiens RepID=MK67I_HUMAN	Q9BYG3 (+1)	34 kDa	2
Putative uncharacterized protein HNRNPA2B1 n=1 Tax=Homo sapiens RepID=B8ZZ37_HUMAN	B8ZZ37 (+1)	34 kDa	2
Putative uncharacterized protein HNRNPAB n=1 Tax=Homo sapiens RepID=B5MD22_HUMAN	B5MD22 (+6)	36 kDa	2
Pyruvate kinase isozymes M1/M2 n=2 Tax=Hominidae RepID=KPYM_HUMAN	P14618	58 kDa	2
Replication factor C 2 isoform 1 (Fragment) n=1 Tax=Homo sapiens RepID=B5BUD2_HUMAN	B5BUD2 (+1)	39 kDa	2
Ribosomal RNA-processing protein 7 homolog A n=1 Tax=Homo sapiens RepID=RRP7A_HUMAN	Q9Y3A4	32 kDa	2
Sialic acid synthase n=1 Tax=Homo sapiens RepID=SIAS_HUMAN	Q9NR45	40 kDa	2
Transcription factor E2F6 n=1 Tax=Homo sapiens RepID=E2F6_HUMAN	O75461 (+3)	32 kDa	2

Truncated nucleolar phosphoprotein B23 n=1 Tax=Homo sapiens RepID=A4ZU86_HUMAN	A4ZU86 (+3)	30 kDa	2
Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C_HUMAN	P68371 (+1)	50 kDa	2