ENCODE DCC Antibody Validation Document

Date of Submission: May 16, 2011

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Antibody Name: Elf-1 (C-20)

Target: ELF1

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-631

Lot Number: L2707

Antibody Description: Rabbit polyclonal, epitope mapping at C-terminus of ELF-1 of human origin

Target Description: E74-like factor 1 (ets domain transcription factor); ~67 kDa
Entrez gene: 1997

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-631-elf-1-

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:

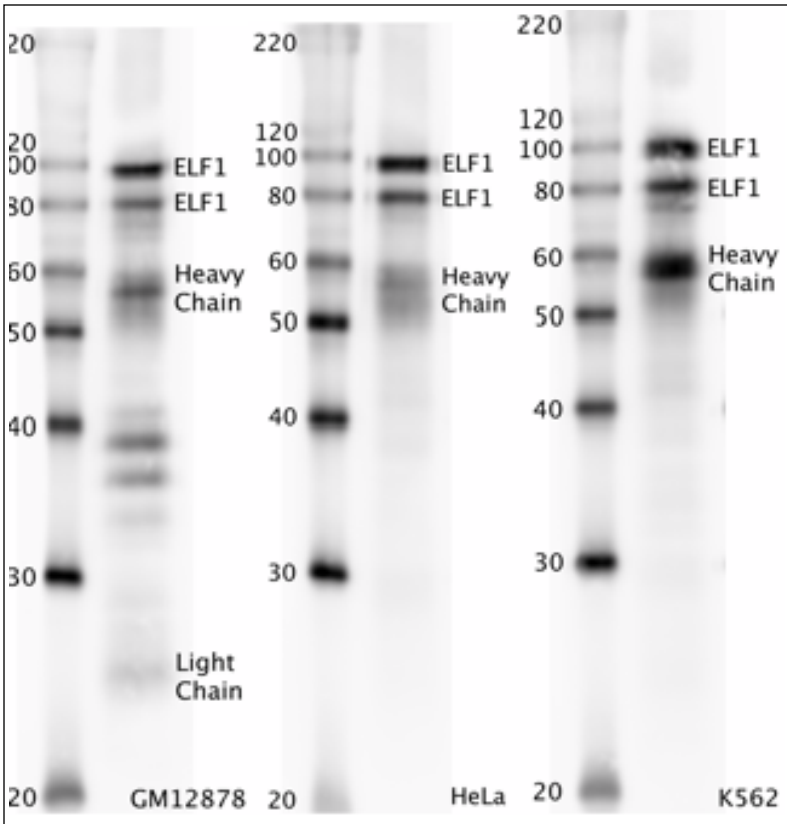
Modification:

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-631 in whole cell lysate of GM12878, HeLa, and K562. ELF1 bands are indicated, as are heavy and light chains of IgG.



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 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 16 for lower band and hit 6 for upper band (targets are highlighted in bold font).

Insert Validation Image (Click here)

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MS results for lower band (~80 kDa):

Identified Proteins (334)	Accession Number	ELF1-1
ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=3	DDX3X_HUMAN	19
Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6	K2C1_HUMAN	18
Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1	HSP7C_HUMAN	14
Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6	K1C10_HUMAN	13
78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2	GRP78_HUMAN	13
Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2	K22E_HUMAN	12
Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4	HS90B_HUMAN	11
Moesin OS=Homo sapiens GN=MSN PE=1 SV=3	MOES_HUMAN	10
Calpain-1 catalytic subunit OS=Homo sapiens GN=CAPN1 PE=1 SV=1	CAN1_HUMAN	9
6-phosphofructokinase, liver type OS=Homo sapiens GN=PFKL PE=1 SV=6	K6PL_HUMAN	8
Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3	K1C9_HUMAN	6
Fermitin family homolog 3 OS=Homo sapiens GN=FERMT3 PE=1 SV=1	URP2_HUMAN	6
Interferon-induced GTP-binding protein Mx1 OS=Homo sapiens GN=MX1 PE=1 SV=4	MX1_HUMAN	6
RNA-binding protein 14 OS=Homo sapiens GN=RBM14 PE=1 SV=2	RBM14_HUMAN	6
Tyrosine-protein kinase BTK OS=Homo sapiens GN=BTK PE=1 SV=3	BTK_HUMAN	6
ETS-related transcription factor Elf-1 OS=Homo sapiens GN=ELF1 PE=1 SV=2	**ELF1_HUMAN**	**5**
Trifunctional enzyme subunit alpha, mitochondrial OS=Homo	ECHA_HUMAN	5

sapiens GN=HADHA PE=1 SV=2		
Protein kinase C beta type OS=Homo sapiens GN=PRKCB PE=1 SV=4	KPCB_HUMAN	5
Nucleolar RNA helicase 2 OS=Homo sapiens GN=DDX21 PE=1 SV=5	DDX21_HUMAN	4
Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3	HNRPM_HUMAN	4
Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2	GRP75_HUMAN	4
2'-5'-oligoadenylate synthase 2 OS=Homo sapiens GN=OAS2 PE=1 SV=3	OAS2_HUMAN	4
Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4	MYH9_HUMAN	3
DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4	MCM7_HUMAN	3
X-ray repair cross-complementing protein 6 OS=Homo sapiens GN=XRCC6 PE=1 SV=2	XRCC6_HUMAN	3
Signal transducer and activator of transcription 1-alpha/beta OS=Homo sapiens GN=STAT1 PE=1 SV=2	STAT1_HUMAN	3
1,4-alpha-glucan-branching enzyme OS=Homo sapiens GN=GBE1 PE=1 SV=2	GLGB_HUMAN	3
Protein kinase C delta type OS=Homo sapiens GN=PRKCD PE=1 SV=2	KPCD_HUMAN	3
Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3	TRAP1_HUMAN	2

MS results for upper band (~100 kDa):

Identified Proteins (334)	Accession Number	ELF1-2
Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1	ENPL_HUMAN	19
C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens GN=MTHFD1 PE=1 SV=3	C1TC_HUMAN	17
Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4	MYH9_HUMAN	16
Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4	EF2_HUMAN	14

Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6	K2C1_HUMAN	12
ETS-related transcription factor Elf-1 OS=Homo sapiens GN=ELF1 PE=1 SV=2	**ELF1_HUMAN**	**8**
Coatomer subunit beta OS=Homo sapiens GN=COPB1 PE=1 SV=3	COPB_HUMAN	8
Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2	K22E_HUMAN	7
Coatomer subunit gamma OS=Homo sapiens GN=COPG PE=1 SV=1	COPG_HUMAN	7
Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6	K1C10_HUMAN	6
Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3	K1C9_HUMAN	6
DNA replication licensing factor MCM6 OS=Homo sapiens GN=MCM6 PE=1 SV=1	MCM6_HUMAN	5
Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4	TERA_HUMAN	5
Splicing factor, proline- and glutamine-rich OS=Homo sapiens GN=SFPQ PE=1 SV=2	SFPQ_HUMAN	5
Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4	HS90B_HUMAN	4
Staphylococcal nuclease domain-containing protein 1 OS=Homo sapiens GN=SND1 PE=1 SV=1	SND1_HUMAN	4
Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens GN=UBA1 PE=1 SV=3	UBA1_HUMAN	4
ATP-dependent RNA helicase DDX54 OS=Homo sapiens GN=DDX54 PE=1 SV=2	DDX54_HUMAN	4
Puromycin-sensitive aminopeptidase OS=Homo sapiens GN=NPEPPS PE=1 SV=2	PSA_HUMAN	4
Myosin-Ig OS=Homo sapiens GN=MYO1G PE=1 SV=1	MYO1G_HUMAN	3
26S proteasome non-ATPase regulatory subunit 2 OS=Homo sapiens GN=PSMD2 PE=1 SV=3	PSMD2_HUMAN	3
DNA replication licensing factor MCM4 OS=Homo sapiens GN=MCM4 PE=1 SV=5	MCM4_HUMAN	3
Hexokinase-1 OS=Homo sapiens GN=HK1 PE=1 SV=3	HXK1_HUMAN	3

Methionyl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=MARS PE=1 SV=2	SYMC_HUMAN	2
Exportin-2 OS=Homo sapiens GN=CSE1L PE=1 SV=3	XPO2_HUMAN	2
Coronin-7 OS=Homo sapiens GN=CORO7 PE=1 SV=2	CORO7_HUMAN	2