ENCODE DCC Antibody Validation Document

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Antibody Name: LEF1_(SC-8592)

Target: LEF1

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-8592

Lot Number: C2409

Antibody Description: Goat polyclonal IgG, epitope mapping near the C-terminus of LEF1 of human origin

Target Description: This gene encodes a transcription factor belonging to a family of proteins that share homology with the high mobility group protein-1. The proteins encoded by this gene can bind to a functionally important site in the T-cell receptor-alpha enhancer, thereby conferring maximal enhancer activity (RefSeq).
Entrez gene ID 51176; 54 kDa

Species Target: Human

Species Host: Goat

Validation Method #1: Western Blot

Validation Method #2: IP-Mass Spec

Purification Method:

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://www.scbt.com/datasheet-8592-lef-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

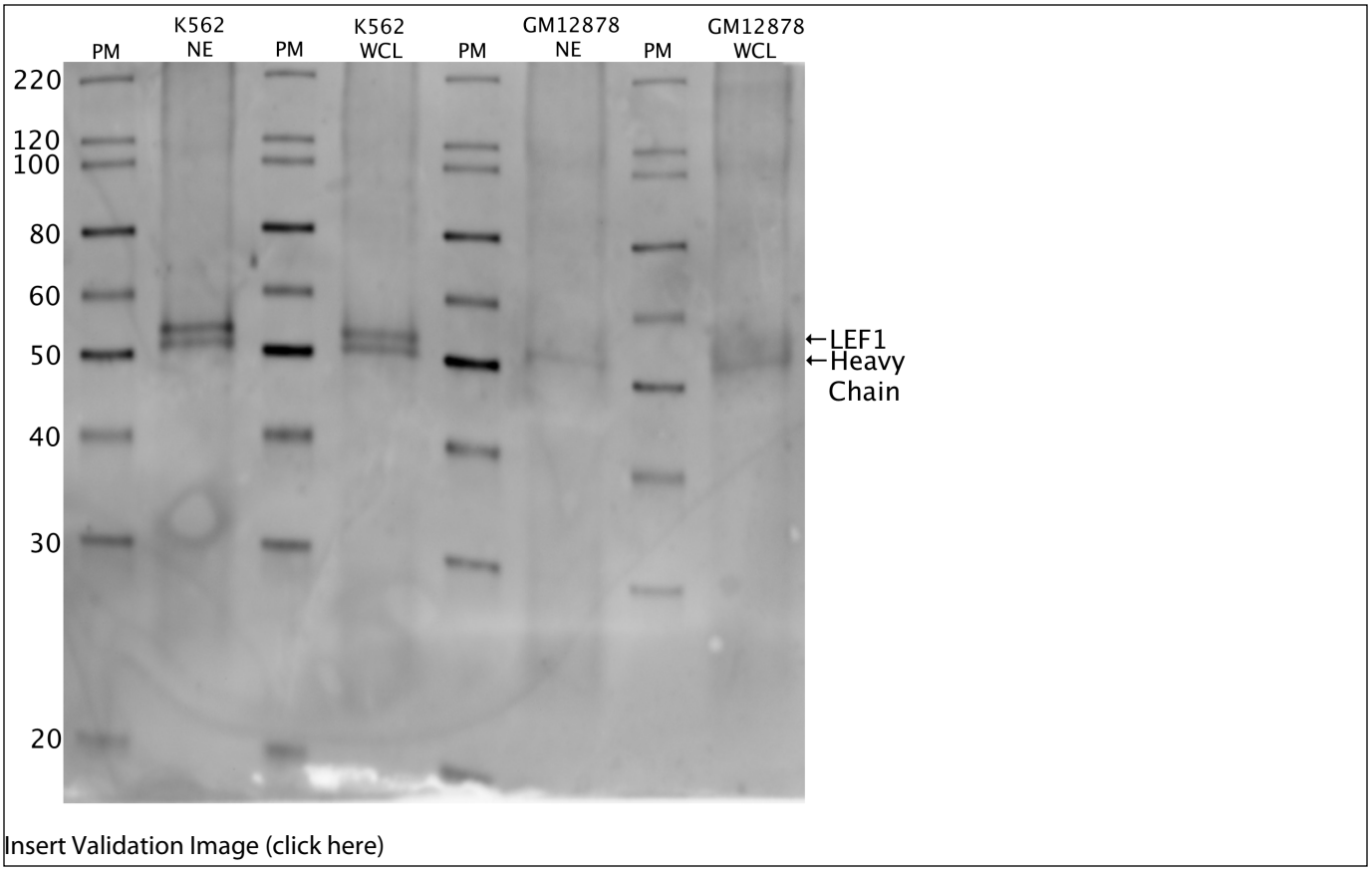
Histone Name | AA modified | AA Position | Modification

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-8592 in nuclear extract (NE) and whole cell lysate (WCL) of K562 and GM12878; PM=protein marker. LEF1 bands are indicated.



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. 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 SEQUEST tandem mass spectra analysis, with probability based matching at $p < 0.05$. As per ENCODE data standards, all SEQUEST results are attached (ENCODE_HAIB_LEF1_sc8592 _09122011_MassSpec.pdf), including common contaminants. Target protein is listed as hit 8 in the ~54 kDa band.

Insert Validation Image (Click here)

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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 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 1.7% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

1. Keratin, type I cytoskeletal 16 n=1 Tax=Homo sapiens RepID=K1C16_HUMAN P08779
2. Serum albumin n=1 Tax=Bos taurus RepID=ALBU_BOVIN P02769
3. Keratin, type II cytoskeletal 5 n=1 Tax=Homo sapiens RepID=K2C5_HUMAN P13647
4. cDNA FLJ32131 fis, clone PEBLM2000267, highly similar to Tubulin alpha-ubiquitous chain n=1 Tax=Homo sapiens RepID=B3KPS3_HUMAN B3KPS3 (+2)
5. Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C_HUMAN P68371 (+2)
6. Keratin, type I cytoskeletal 17 n=1 Tax=Homo sapiens RepID=K1C17_HUMAN Q04695
7. Keratin, type I cytoskeletal 14 n=1 Tax=Homo sapiens RepID=K1C14_HUMAN P02533
8. **Lymphoid enhancer-binding factor 1, isoform CRA_c n=1 Tax=Homo sapiens RepID=B4DG38_HUMAN B4DG38 (+1)**
9. Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA_HUMAN P06733
10. Tubulin beta chain n=12 Tax=Amniota RepID=TBB5_HUMAN P07437
11. ATP synthase subunit alpha, mitochondrial n=3 Tax=Hominidae RepID=ATPA_HUMAN P25705
12. Keratin, type II cytoskeletal 6C n=1 Tax=Homo sapiens RepID=K2C6C_HUMAN P48668 (+1)
13. cDNA FLJ52842, highly similar to Actin, cytoplasmic 1 n=1 Tax=Homo sapiens RepID=B4E335_HUMAN B4E335 (+7)
14. Junction plakoglobin n=1 Tax=Homo sapiens RepID=PLAK_HUMAN P14923
15. Hornerin n=1 Tax=Homo sapiens RepID=Q5DT20_HUMAN Q5DT20 (+1)