ENCODE DCC Antibody Validation Document

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Antibody Name: SP1

Target: SP1

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-17824

Lot Number: K1907

Antibody Description: Sp1(E-3) is a mouse monoclonal antibody raised against amino acids 121-345 mapping near the N-terminus of Sp1 ofhuman origin.

Target Description: SP1 is a transcription factor that binds to GC box promoters elements and selectively activates mRNA synthesis from genes that contain functional recognition sites. It can interact with G/C-rich motifs from the serotonin receptor promoter.
Entrez gene ID 6667; 106 kDa

Species Target: Human

Species Host: Mouse

Validation Method #1: Western Blot

Validation Method #2: IP-Mass Spec

Purification Method:

Polyclonal/ Monoclonal: Monoclonal

Vendor URL: http://www.scbt.com/datasheet-17824-sp1-

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

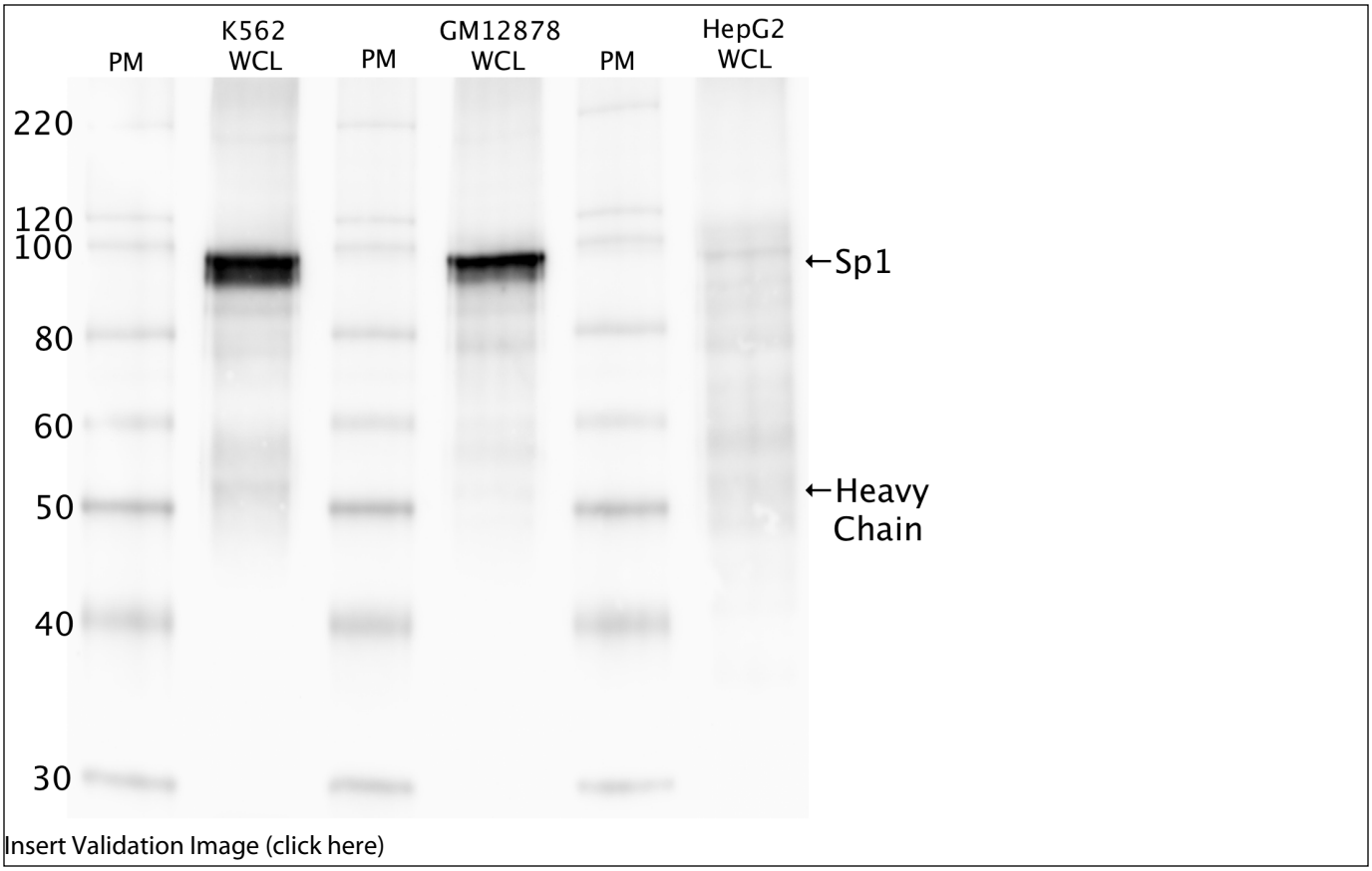
Histone Name | AA modified | AA Position | Modification

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-17824 in whole cell lysate (WCL) of K562, GM12878 and HepG2; PM=protein marker. SP1 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_SP1_sc17824_09122011_MassSpec.pdf), including common contaminants. Target protein is listed as hit 25 in ~100 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 0.0% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

1. Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 HS90B_HUMAN
2. Nucleolar RNA helicase 2 OS=Homo sapiens GN=DDX21 PE=1 SV=5 DDX21_HUMAN
3. DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4 MCM7_HUMAN
4. TBC1 domain family member 5 OS=Homo sapiens GN=TBC1D5 PE=1 SV=1TBCD5_HUMAN
5. Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4 EF2_HUMAN
6. Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5 HS90A_HUMAN
7. Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1 ENPL_HUMAN
8. DNA replication licensing factor MCM5 OS=Homo sapiens GN=MCM5 PE=1 SV=5 MCM5_HUMAN
9. Programmed cell death 6-interacting protein OS=Homo sapiens GN=PDCD6IP PE=1 SV=1 PDC6I_HUMAN
10. Methionyl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=MARS PE=1 SV=2 SYMC_HUMAN
11. Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4 TERA_HUMAN
12. 26S proteasome non-ATPase regulatory subunit 2 OS=Homo sapiens GN=PSMD2 PE=1 SV=3 PSMD2_HUMAN
13. Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3 GANAB_HUMAN
14. Transferrin receptor protein 1 OS=Homo sapiens GN=TFRC PE=1 SV=2 TFR1_HUMAN

15. X-ray repair cross-complementing protein 5 OS=Homo sapiens GN=XRCC5 PE=1 SV=3 XRCC5_HUMAN
16. Transportin-3 OS=Homo sapiens GN=TNPO3 PE=1 SV=3 TNPO3_HUMAN
17. Coatomer subunit gamma OS=Homo sapiens GN=COPG PE=1 SV=1 COPG_HUMAN
18. Probable ATP-dependent RNA helicase DDX27 OS=Homo sapiens GN=DDX27 PE=1 SV=2 DDX27_HUMAN
19. Transportin-1 OS=Homo sapiens GN=TNPO1 PE=1 SV=2 TNPO1_HUMAN
20. Coatomer subunit gamma-2 OS=Homo sapiens GN=COPG2 PE=1 SV=1 COPG2_HUMAN
21. ATP-dependent RNA helicase DDX1 OS=Homo sapiens GN=DDX1 PE=1 SV=2 DDX1_HUMAN
22. Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 K2C1_HUMAN
23. 6-phosphofructokinase type C OS=Homo sapiens GN=PFKP PE=1 SV=2 K6PP_HUMAN
24. N-alpha-acetyltransferase 15, NatA auxiliary subunit OS=Homo sapiens GN=NAA15 PE=1 SV=1 NAA15_HUMAN
25. **Transcription factor Sp1 OS=Homo sapiens GN=SP1 PE=1 SV=3 SP1_HUMAN**
26. Signal transducer and activator of transcription 5A OS=Homo sapiens GN=STAT5A PE=1 SV=1 STA5A_HUMAN