ENCODE DCC Antibody Validation Document

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Antibody Name: USF-1

Target: USF-1 (C-20)

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-229

Lot Number: A2109

Antibody Description: Rabbit polyclonal, USF-1 (C-20)

Target Description: The ubiquitously expressed cellular upstream stimulatory factor (USF) consists of USF-1 and USF-2 polypeptides which independently exhibit site-specific DNA binding and are members of the c-Myc-related family of regulatory factors containing helix-loop-helix domains.
Entrez gene ID 7391; 43 kDa

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-229-usf-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

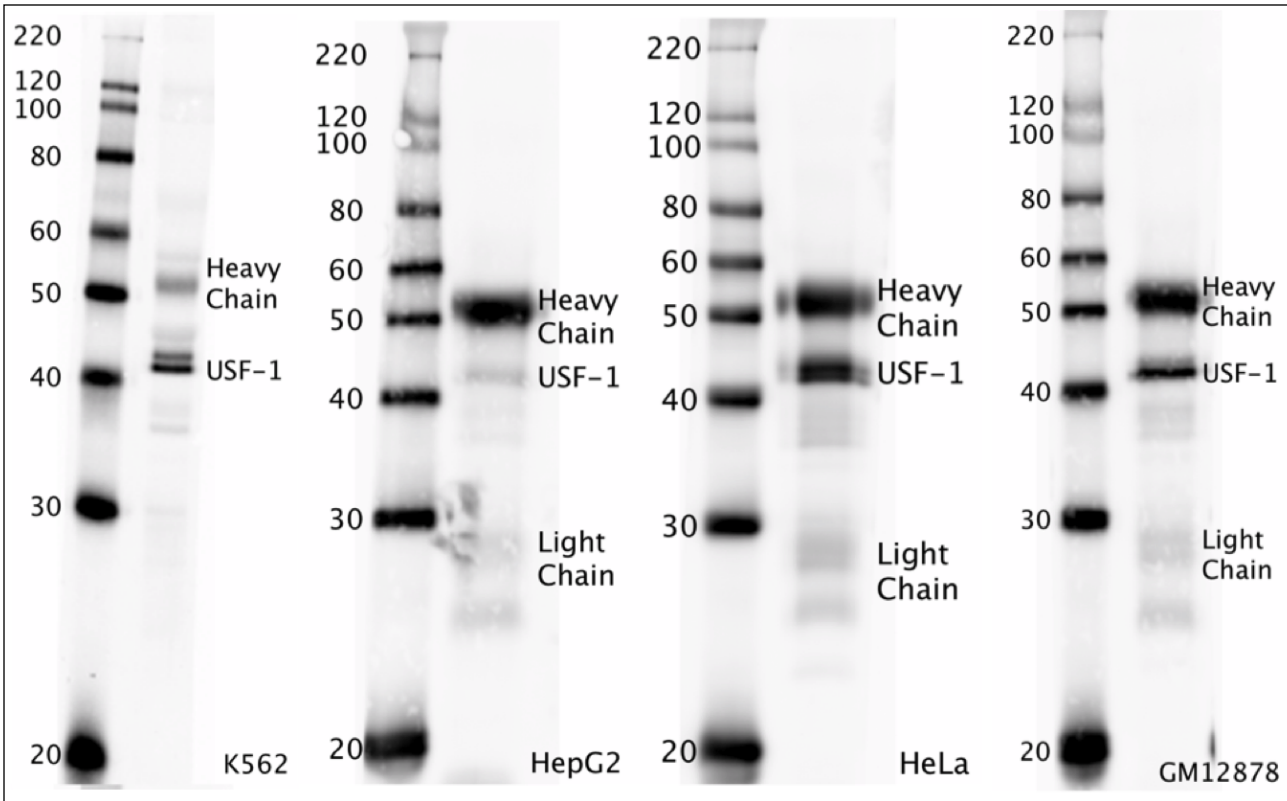
Histone Name | AA modified | AA Position | Modification

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-229 in whole cell lysate (WCL) of HeLa, HepG2, K562 and GM12878 ; PM=protein marker. USF1 bands are indicated.



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 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_USF1_sc229_09122011_MassSpec.pdf), including common contaminants. Target protein is listed as hit 23 in the ~43 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, 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 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. Phosphoglycerate kinase 1 OS=Homo sapiens GN=PGK1 PE=1 SV=3 PGK1_HUMAN
2. Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 ACTB_HUMAN (+1)
3. Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM2 PE=1 SV=4KPYM_HUMAN
4. Acetyl-CoA acetyltransferase, mitochondrial OS=Homo sapiens GN=ACAT1 PE=1 SV=1 THIL_HUMAN
5. Upstream stimulatory factor 2 OS=Homo sapiens GN=USF2 PE=1 SV=1 USF2_HUMAN
6. Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2 ALDOA_HUMAN
7. Actin-related protein 2 OS=Homo sapiens GN=ACTR2 PE=1 SV=1 ARP2_HUMAN
8. Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1 EF1A1_HUMAN
9. Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 ENOA_HUMAN
10. Developmentally-regulated GTP-binding protein 1 OS=Homo sapiens GN=DRG1 PE=1 SV=1 DRG1_HUMAN
11. Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial OS=Homo sapiens GN=IDH3B PE=1 SV=2 IDH3B_HUMAN
12. Medium-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Homo sapiens GN=ACADM PE=1 SV=1 ACADM_HUMAN
13. Fructose-bisphosphate aldolase C OS=Homo sapiens GN=ALDOC PE=1 SV=2 ALDOC_HUMAN
14. ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3 ATPB_HUMAN

15. 26S protease regulatory subunit 10B OS=Homo sapiens GN=PSMC6 PE=1 SV=1 PRS10_HUMAN
16. 40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4 RSSA_HUMAN
17. HLA class I histocompatibility antigen, Cw-4 alpha chain OS=Homo sapiens GN=HLA-C PE=1 SV=1 1C04_HUMAN (+2)
18. Heterogeneous nuclear ribonucleoprotein D0 OS=Homo sapiens GN=HNRNPD PE=1 SV=1 HNRPD_HUMAN
19. Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 HS90B_HUMAN
20. Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial OS=Homo sapiens GN=IDH3A PE=1 SV=1 IDH3A_HUMAN
21. Stomatin-like protein 2 OS=Homo sapiens GN=STOML2 PE=1 SV=1 STML2_HUMAN
22. Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2 TBB5_HUMAN
23. **Upstream stimulatory factor 1 OS=Homo sapiens GN=USF1 PE=1 SV=1 USF1_HUMAN**