ENCODE Antibody Validation Documentation
Transcription factor: USF1 upstream transcription factor 1 (GeneID 7391)

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Transcription factor: USF1 (GeneID 7391; ~33 kDa)

Antibody: USF-1 (C-20), Santa Cruz Biotechnology (sc-229)
Rabbit polyclonal, epitope mapping at the C-terminus of USF-1 of human origin
Web: http://www.scbt.com/datasheet-229-usf-1-c-20-antibody.html

Validation 1: Immunoblot Analysis

For an antibody to meet ENCODE validation standards, a single band of the predicted size, or a band of no less than half the total signal, must be detected in a lane on a Western blot.

a. Vendor immunoblot analysis

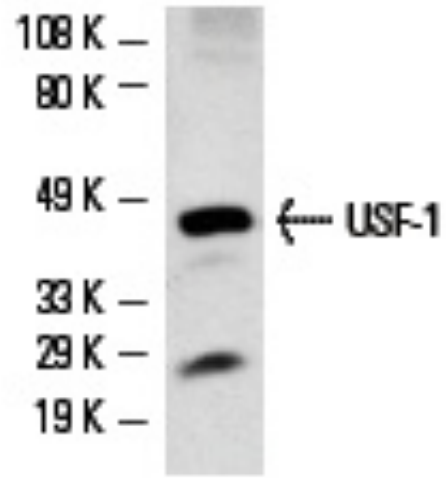

Figure Legend: Western blot analysis of USF-1 expression in NIH/3T3 nuclear extract.

b. Myers Lab immunoblot analysis

Western blot protocol

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. After separation, the samples were transferred to a nitrocellulose membrane using a Bio-Rad Trans-Blot Electrophoretic Transfer system. Standard western blot protocol was used to probe the membrane with the primary antibody (same antibody as used for IP), and an HRP-conjugated secondary antibody and SuperSignal West Femto solution (Thermo Scientific) were used to detect the immunoprecipitated proteins.

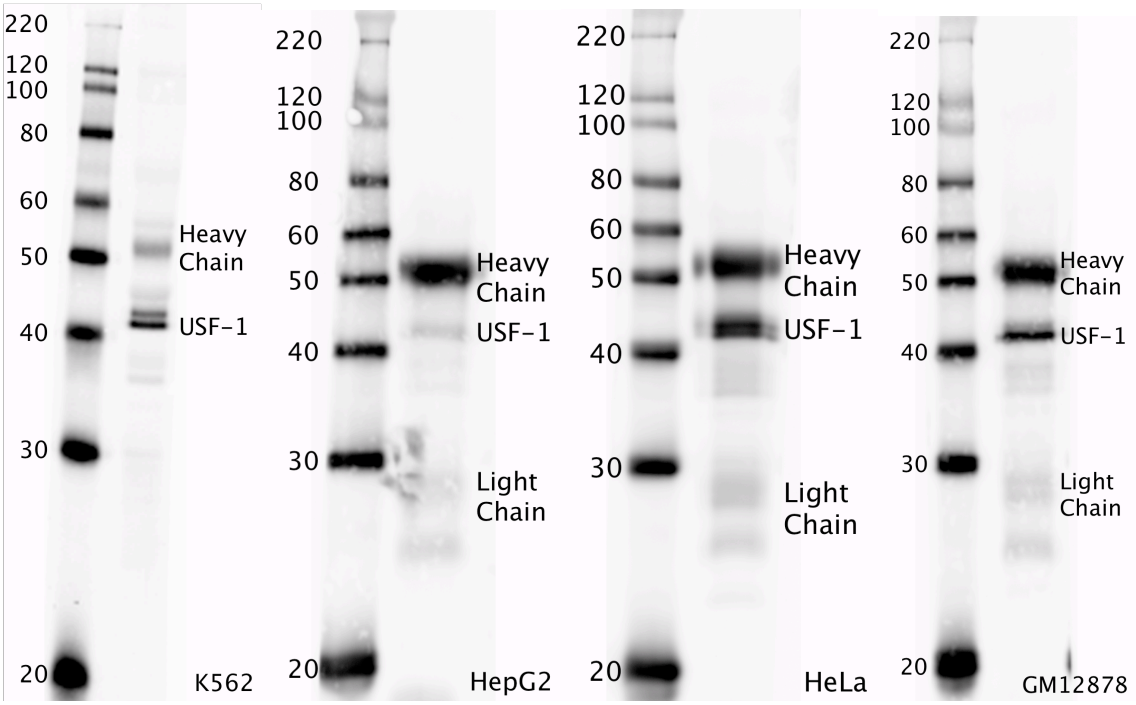

Figure Legend: USF1 immunoblot: IP-western with sc-229 USF1 antibody in whole cell lysates of, left to right: K562, HepG2, HeLa, and GM12878. Heavy chain and light chain of IgG are indicated, and USF1 band is indicated at ~43 kDa.

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.

Phosphoglycerate kinase 1 OS=Homo sapiens GN=PGK1 PE=1 SV=3 PGK1_HUMAN

Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 ACTB_HUMAN (+1)

Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM2 PE=1 SV=4 KPYM_HUMAN

Acetyl-CoA acetyltransferase, mitochondrial OS=Homo sapiens GN=ACAT1 PE=1 SV=1 THIL_HUMAN

Upstream stimulatory factor 2 OS=Homo sapiens GN=USF2 PE=1 SV=1 USF2_HUMAN

Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2 ALDOA_HUMAN

Actin-related protein 2 OS=Homo sapiens GN=ACTR2 PE=1 SV=1 ARP2_HUMAN

Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1 EF1A1_HUMAN

Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 ENOA_HUMAN

Developmentally-regulated GTP-binding protein 1 OS=Homo sapiens GN=DRG1 PE=1 SV=1 DRG1_HUMAN

Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial OS=Homo sapiens GN=IDH3B PE=1 SV=2 IDH3B_HUMAN

Medium-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Homo sapiens GN=ACADM PE=1 SV=1 ACADM_HUMAN

Fructose-bisphosphate aldolase C OS=Homo sapiens GN=ALDOC PE=1 SV=2 ALDOC_HUMAN

ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3 ATPB_HUMAN

26S protease regulatory subunit 10B OS=Homo sapiens GN=PSMC6 PE=1 SV=1 PRS10_HUMAN

40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4 RSSA_HUMAN

HLA class I histocompatibility antigen, Cw-4 alpha chain OS=Homo sapiens GN=HLA-C PE=1 SV=1 1C04_HUMAN (+2)

Heterogeneous nuclear ribonucleoprotein D0 OS=Homo sapiens GN=HNRNPD PE=1 SV=1 HNRPD_HUMAN

Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 HS90B_HUMAN

Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial OS=Homo sapiens GN=IDH3A PE=1 SV=1 IDH3A_HUMAN

Stomatin-like protein 2 OS=Homo sapiens GN=STOML2 PE=1 SV=1 STML2_HUMAN

Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2 TBB5_HUMAN

Upstream stimulatory factor 1 OS=Homo sapiens GN=USF1 PE=1 SV=1USF1_HUMAN