ENCODE Antibody Validation Documentation
Transcription factor: ETS1 (GeneID 2113)

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Transcription factor: ETS1 (GeneID 2113; ~55 kDa)

Antibody: ETS1 (C-20), Santa Cruz Biotechnology (sc-350)
Rabbit polyclonal, epitope mapping at the C-terminus of ETS1 of human origin
Web: http://www.scbt.com/datasheet-350-ets-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

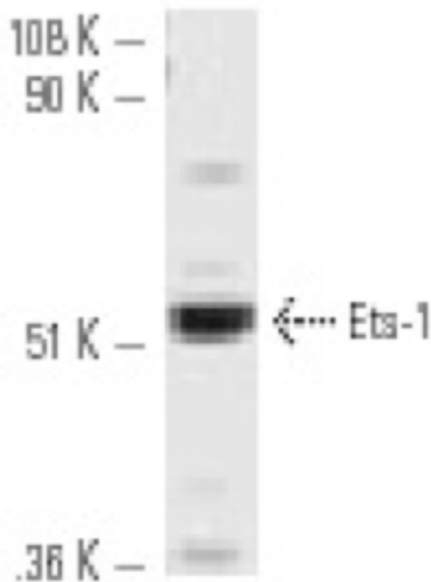

Figure Legend: Western blot analysis of ETS1 expression in KNRK nuclear extract.

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 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).

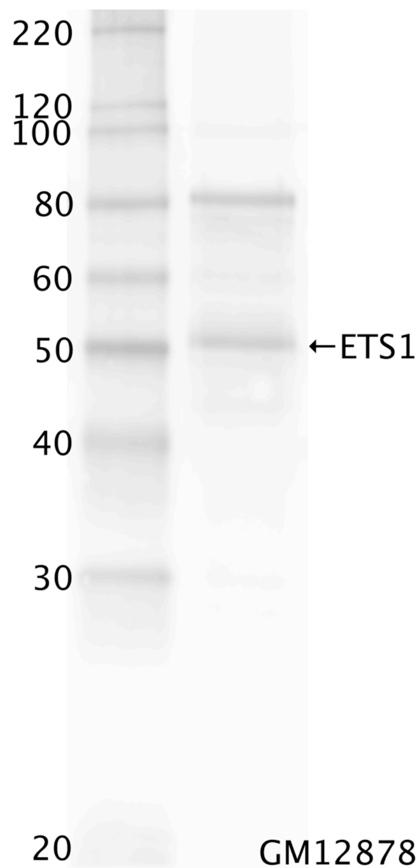

Figure Legend: ETS1 immunoblot: IP-western with sc-350 ETS1 antibody in whole cell lysate of GM12878. ETS1 band is indicated at ~52 kDa.

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 at ~52 kDa 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. [No match for ETS1 was found by mass spec analysis in the band at ~80 kDa.]

ATP synthase subunit alpha, mitochondrial n=3 Tax=Homininae RepID=ATPA_HUMAN P25705 13

Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C_HUMAN P68371 (+2) 13

ATP synthase subunit beta, mitochondrial n=1 Tax=Homo sapiens RepID=ATPB_HUMAN P06576 12

Aspartyl-tRNA synthetase, cytoplasmic n=4 Tax=Homo sapiens RepID=SYDC_HUMAN P14868 11

cDNA FLJ32131 fis, clone PEBLM2000267, highly similar to Tubulin alpha-ubiquitous chain n=1 Tax=Homo sapiens RepID=B3KPS3_HUMAN B3KPS3 (+2) 9

Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA_HUMAN P06733 9

cDNA FLJ52842, highly similar to Actin, cytoplasmic 1 n=1 Tax=Homo sapiens RepID=B4E335_HUMAN B4E335 (+7) 5

Heterogeneous nuclear ribonucleoprotein H, N-terminally processed n=2 Tax=Homo sapiens RepID=HNRH1_HUMAN P31943 (+1) 4

cDNA FLJ16143 fis, clone BRAMY2038516, highly similar to Protein disulfide-isomerase A6 (EC 5.3.4.1) n=1 Tax=Homo sapiens RepID=B3KY95_HUMAN B3KY95 (+4) 3

cDNA FLJ59231, highly similar to C-ets-1 protein n=1 Tax=Homo sapiens RepID=B4DW78_HUMAN B4DW78 (+2) 3

Tubulin beta chain n=12 Tax=Amniota RepID=TBB5_HUMAN P07437 3