ENCODE Antibody Validation Documentation
Transcription factor: Interferon regulatory factor 4 (GeneID 3662)

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Transcription factor: IRF4 (GeneID 3662; ~52 kDa)

Antibody: IRF4 (M-17), Santa Cruz Biotechnology (sc-6059)
Goat polyclonal, epitope mapping at C-terminus of IRF4 of mouse origin
Web: http://www.scbt.com/datasheet-6059-irf-4-m-17-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 IRF4 expression in MM-142 (A), HuT 78 (B) and Ramos (C) whole cell lysates.
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:** IRF4 immunoblot: IP-western with sc-6059 IRF4 antibody in whole cell lysates (WCL) of GM12878 and K562. IRF4 band is indicated at ~50 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 Coomassie 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 1.8% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

ATP synthase subunit beta, mitochondrial n=1 Tax=Homo sapiens RepID=ATPB_HUMAN P06576 (+1)
cDNA FLJ52842, highly similar to Actin, cytoplasmic 1 n=1 Tax=Homo sapiens RepID=B4E335_HUMAN B4E335 (+7)
Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA_HUMAN P06733
Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C_HUMAN P68371 (+1)
Pyruvate kinase isozymes M1/M2 n=2 Tax=Homininae RepID=KPYM_HUMAN P14618
Interferon regulatory factor 4 n=1 Tax=Homo sapiens RepID=IRF4_HUMAN Q15306 (+1)
cDNA FLJ78244, highly similar to Homo sapiens eukaryotic translation initiation factor 4A, isoform 1 (EIF4A1), mRNA n=1 Tax=Homo sapiens RepID=A8K7F6_HUMAN A8K7F6 (+1)
ATP synthase subunit alpha, mitochondrial n=3 Tax=Homininae RepID=ATPA_HUMAN P25705
6-phosphogluconate dehydrogenase, decarboxylating n=3 Tax=Homo sapiens RepID=6PGD_HUMAN P52209
cDNA FLJ78120, highly similar to Homo sapiens eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa (EIF2S3), mRNA n=1 Tax=Homo sapiens RepID=A8K2Y2_HUMAN A8K2Y2 (+2)
cDNA FLJ75299, highly similar to Xenopus laevis proteasome (prosome, macropain) 26S subunit, ATPase 3, mRNA n=1 Tax=Homo sapiens RepID=A8K781_HUMAN A8K781 (+1)
RuvB-like 2 (E. coli), isoform CRA_d n=1 Tax=Homo sapiens RepID=B3KNL2_HUMAN B3KNL2 (+2)
60S ribosomal protein L4 n=1 Tax=Homo sapiens RepID=RL4_HUMAN P36578 (+2)
60S ribosomal protein L3 n=1 Tax=Homo sapiens RepID=RL3_HUMAN P39023 (+4)
Tubulin alpha-1C chain n=2 Tax=Homininae RepID=TBA1C_HUMAN Q9BQE3

cDNA FLJ78579, highly similar to Homo sapiens c-src tyrosine kinase (CSK), mRNA n=1 Tax=Homo sapiens RepID=A8K3B6_HUMAN A8K3B6 (+4)

cDNA FLJ75185 n=1 Tax=Homo sapiens RepID=A8K3D0_HUMAN A8K3D0 (+10)

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)

cDNA FLJ52929, highly similar to Dolichyl-diphosphooligosaccharide--proteinglycosyltransferase 48 kDa subunit (EC2.4.1.119) n=1 Tax=Homo sapiens RepID=B4DJE3_HUMAN B4DJE3 (+3)

cDNA FLJ60299, highly similar to Rab GDP dissociation inhibitor beta n=1 Tax=Homo sapiens RepID=B4DLV7_HUMAN B4DLV7 (+3)

cDNA FLJ54023, highly similar to Heat shock protein HSP 90-beta n=1 Tax=Homo sapiens RepID=B4DMA2_HUMAN B4DMA2 (+1)

cDNA FLJ56389, highly similar to Elongation factor 1-gamma n=1 Tax=Homo sapiens RepID=B4DTG2_HUMAN B4DTG2 (+1)

D-3-phosphoglycerate dehydrogenase n=1 Tax=Homo sapiens RepID=SERA_HUMAN O43175

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

Elongation factor Tu, mitochondrial n=1 Tax=Homo sapiens RepID=EFTU_HUMAN P49411

Actin-related protein 3 n=4 Tax=Eutheria RepID=ARP3_HUMAN P61158

Tubulin alpha-4A chain n=8 Tax=Eutheria RepID=TBA4A_HUMAN P68366

Putative uncharacterized protein (Fragment) n=1 Tax=Homo sapiens RepID=Q59G88_HUMAN Q59G88 (+1)

keratin, type II cytoskeletal 7 n=1 Tax=Homo sapiens RepID=UPI000013CF9E UPI000013CF9E (+1)