ENCODE Antibody Validation Documentation

Transcription factor: TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55kDa (GeneID 6879)

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Transcription factor: TAF7 (GeneID 6879; ~40 kDa)

Antibody: TAF II p55 (SQ-8), Santa Cruz Biotechnology (sc-101167)
Mouse monoclonal, raised against recombinant TAF II p55 of human origin

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

![Western blot analysis of TAF II p55 expression in MCF7 whole cell lysate.](image)

**Figure Legend:** Western blot analysis of TAF II p55 expression in MCF7 whole cell lysate.
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: TAF7 immunoblot: IP-western with sc-101167 TAF7 antibody in whole cell lysates (WCL) of K562 and GM12878. Heavy chain and light chain of IgG are indicated, and TAF7 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, 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. 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.

Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C_HUMAN P68371 (+1)

Alpha-enolase n=1 Tax=Homo sapiens RepID=ENO A_HUMAN P06733

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

ATP synthase subunit beta, mitochondrial n=1 Tax=Homo sapiens RepID=ATPB_HUMAN P06576 (+1)

Tubulin alpha-1C chain n=2 Tax=Homininae RepID=TBA1C_HUMAN Q9BQE3

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)

RuvB-like 1 (Fragment) n=1 Tax=Homo sapiens RepID=B5BUB1_HUMAN B5BUB1 (+1)

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

Transcription initiation factor TFIID subunit 7 n=3 Tax=Catarrhini RepID=TAF7_HUMAN Q15545