ENCODE DCC Antibody Validation Document

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Antibody Name: TAF7_(SC-101167)

Target: TAF7

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-101167

Lot Number: J1509

Antibody Description: Mouse monoclonal IgG, raised against recombinant TAF II p55 (TAF7) of human origin.

Target Description: The intronless gene for this transcription coactivator is located between the protocadherin beta and gamma gene clusters on chromosome 5. The protein encoded by this gene is a component of the TFIID protein complex.
Entrez gene ID 6879; 55 kDa

Species Target: Human

Species Host: Mouse

Validation Method #1: Western Blot

Validation Method #2: IP-Mass Spec

Purification Method:

Polyclonal/ Monoclonal: Monoclonal

Vendor URL: http://www.scbt.com/datasheet-101167-taf-

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

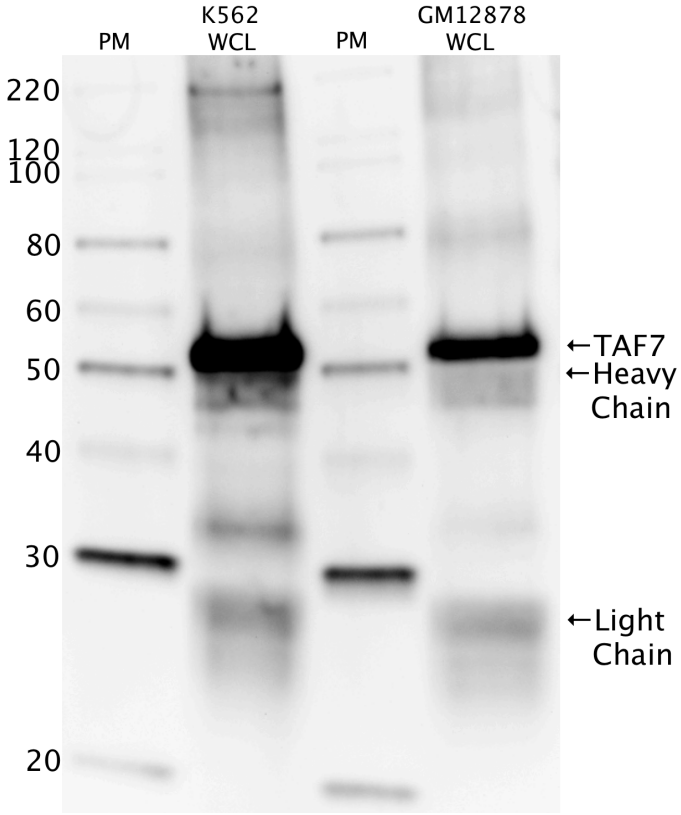
Histone Name: AA modified: AA Position: Modification:

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-101167 in whole cell lysate (WCL) of K562 and Gm12878; PM=protein marker. TAF7 bands are indicated.



Insert Validation Image (click here)

Validation #2 Analysis	IP followed by mass spectrometry: 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. 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_TAF7_sc101167_09122011_MassSpec.pdf), including common contaminants. Target protein is listed as hit 9 in the ~50 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, 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 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 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.

1. Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C_HUMAN P68371 (+1)
2. Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA_HUMAN P06733
3. ATP synthase subunit alpha, mitochondrial n=3 Tax=Homininae RepID=ATPA_HUMAN P25705
4. ATP synthase subunit beta, mitochondrial n=1 Tax=Homo sapiens RepID=ATPB_HUMAN P06576 (+1)
5. Tubulin alpha-1C chain n=2 Tax=Homininae RepID=TBA1C_HUMAN Q9BQE3
6. 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)
7. RuvB-like 1 (Fragment) n=1 Tax=Homo sapiens RepID=B5BUB1_HUMAN B5BUB1 (+1)
8. Aspartyl-tRNA synthetase, cytoplasmic n=4 Tax=Homo sapiens RepID=SYDC_HUMAN P14868
9. **Transcription initiation factor TFIID subunit 7 n=3 Tax=Catarrhini RepID=TAF7_HUMAN Q15545**