

## ENCODE Antibody Validation Documentation

### Transcription factor: MYC associated factor X (GeneID 4149)

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**Transcription factor:** MAX (GeneID 4149; ~18 kDa)

**Antibody:** MAX (C-17), Santa Cruz Biotechnology (sc-197)

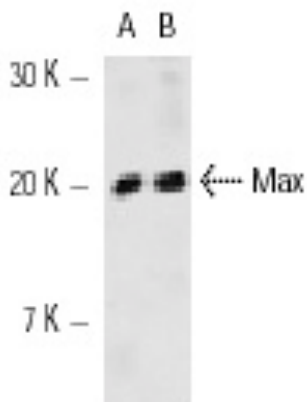
Rabbit polyclonal, epitope mapping at the C-terminus of MAX of human origin

Web: <http://www.scbt.com/datasheet-197-max-c-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

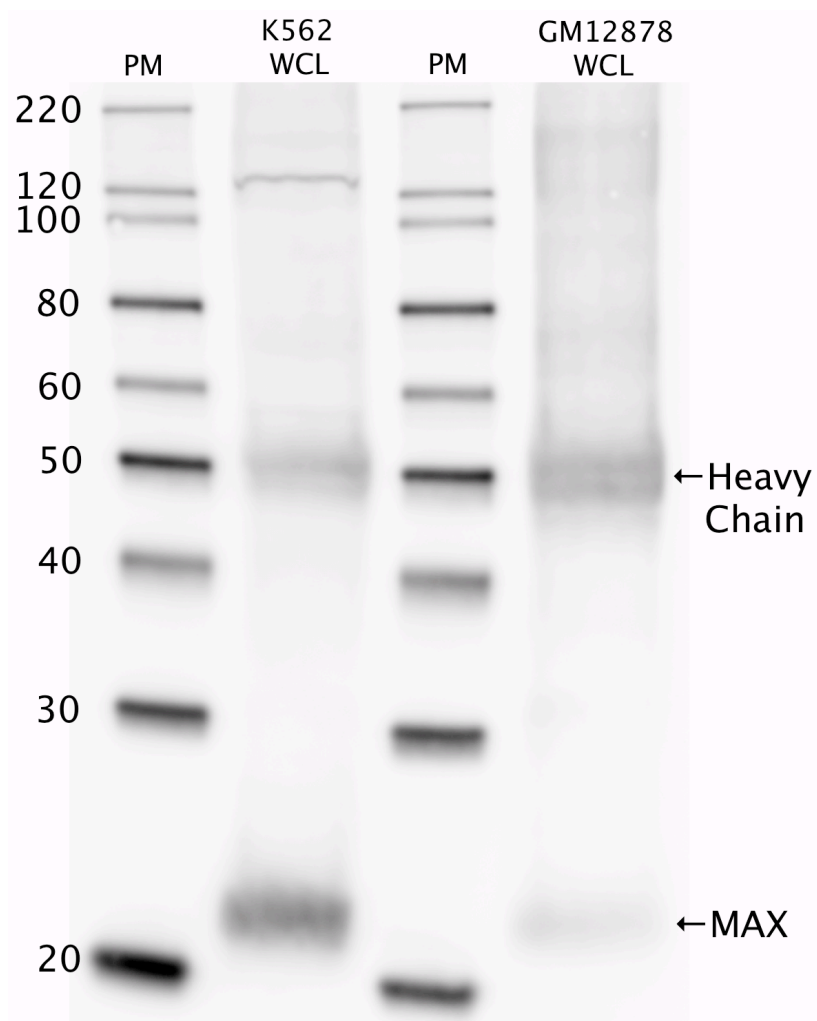


**Figure Legend:** Western blot analysis of MAX expression in HeLa (A) and K562 (B) nuclear extracts.

## 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:** MAX immunoblot: IP-western with sc-197 MAX antibody in whole cell lysates (WCL) of K562 and GM12878. Heavy chain of IgG is indicated, and MAX band is indicated at ~22 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.

Cytochrome c oxidase subunit 2 n=1 Tax=Homo sapiens RepID=A0S2P1\_HUMAN A0S2P1 (+63)

Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C\_HUMAN P68371 (+1)

60S ribosomal protein L18 n=3 Tax=Catarrhini RepID=RL18\_HUMAN Q07020

cDNA FLJ75549, highly similar to Homo sapiens ribosomal protein, large, P0 (RPLP0), transcript variant 1, mRNA n=1 Tax=Homo sapiens RepID=A8K4Z4\_HUMAN A8K4Z4 (+1)

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

Putative uncharacterized protein RAN n=1 Tax=Homo sapiens RepID=B5MDF5\_HUMAN B5MDF5 (+2)

Putative uncharacterized protein RPL24 n=1 Tax=Homo sapiens RepID=C9JNW5\_HUMAN C9JNW5 (+2)

Chloride intracellular channel protein 1 n=2 Tax=Homo sapiens RepID=CLIC1\_HUMAN O00299 (+2)

L-lactate dehydrogenase B chain n=4 Tax=Catarrhini RepID=LDHB\_HUMAN P07195

Tubulin beta chain n=12 Tax=Amniota RepID=TBB5\_HUMAN P07437

60S ribosomal protein L9 n=3 Tax=Homo sapiens RepID=RL9\_HUMAN P32969

60S ribosomal protein L21 n=3 Tax=Eutheria RepID=RL21\_HUMAN P46778

40S ribosomal protein S9 n=13 Tax=Eutheria RepID=RS9\_HUMAN P46781

Signal peptidase complex subunit 3 n=4 Tax=Eutheria RepID=SPCS3\_HUMAN P61009

**Protein max n=2 Tax=Eutheria RepID=MAX\_HUMAN P61244 (+1)**

Chromobox protein homolog 3 n=9 Tax=Eutheria RepID=CBX3\_HUMAN Q13185

Ribosomal protein S8 n=2 Tax=Homo sapiens RepID=Q5JR95\_HUMAN Q5JR95 (+1)