

ENCODE Antibody Validation Documentation
Transcription factor: histone deacetylase 2 (GeneID 3066)

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Transcription factor: HDAC2 (GeneID 3066; ~55 kDa)

Antibody: HDAC2 (C-19), Santa Cruz Biotechnology (sc-6296)

Goat polyclonal, epitope mapping at C-terminus of HDAC2 of human origin

Web: <http://www.scbt.com/datasheet-6296-hdac2-c-19-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

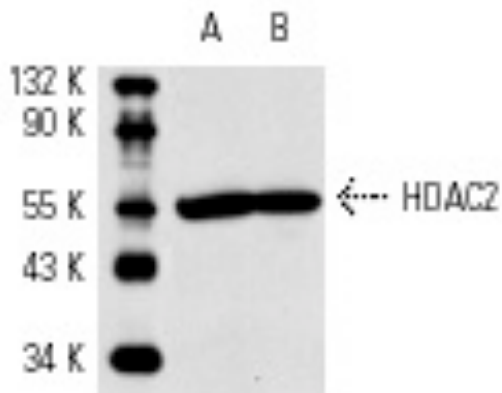


Figure Legend: Western blot analysis of HDAC2 expression in K562 (A) and phorbol-induced Jurkat (B) nuclear extracts.

b. Myers Lab immunoblot analysis

Western blot protocol

Whole cell lysates or nuclear extracts 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).

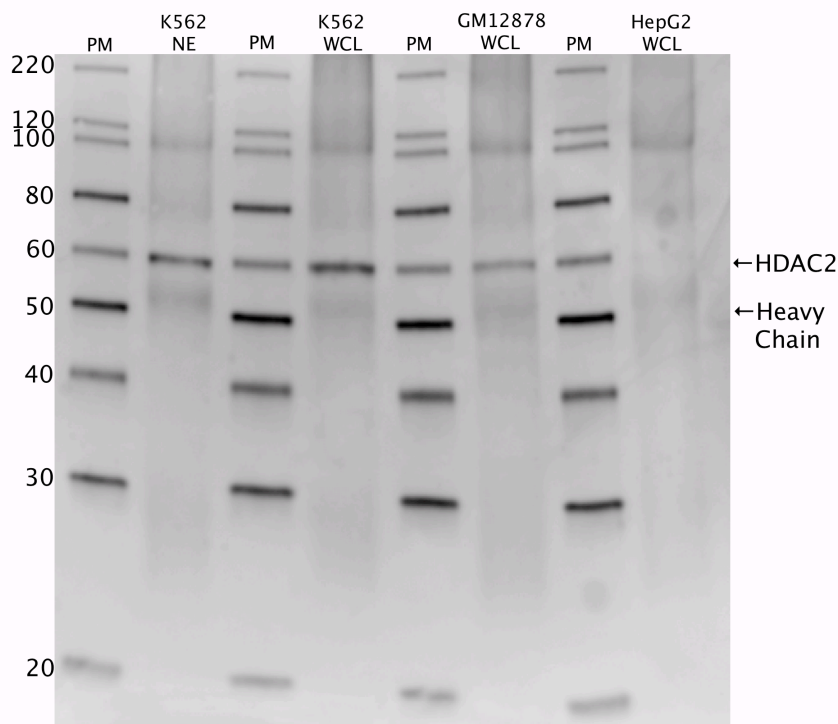


Figure Legend: HDAC2 immunoblot: IP-western with sc-6296 HDAC2 antibody in nuclear extract (NE) or whole cell lysates (WCL) of K562, GM12878, and HepG2. Heavy chain of IgG is indicated, and HDAC2 band is indicated at ~60 kDa.

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 0.0% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

T-complex protein 1 subunit alpha OS=Homo sapiens GN=TCP1 PE=1 SV=1 TCPA_HUMAN

Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4 VIME_HUMAN

T-complex protein 1 subunit beta OS=Homo sapiens GN=CCT2 PE=1 SV=4 TCPB_HUMAN

T-complex protein 1 subunit eta OS=Homo sapiens GN=CCT7 PE=1 SV=2 TCPH_HUMAN

60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2 CH60_HUMAN

Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM2 PE=1 SV=4 KPYM_HUMAN

T-complex protein 1 subunit delta OS=Homo sapiens GN=CCT4 PE=1 SV=4 TCPD_HUMAN

T-complex protein 1 subunit epsilon OS=Homo sapiens GN=CCT5 PE=1 SV=1 TCPE_HUMAN

Tyrosyl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=YARS PE=1 SV=4 SYYC_HUMAN

Asparagine synthetase [glutamine-hydrolyzing] OS=Homo sapiens GN=ASNS PE=1 SV=4 ASNS_HUMAN

Non-POU domain-containing octamer-binding protein OS=Homo sapiens GN=NONO PE=1 SV=4 NONO_HUMAN

Histone deacetylase 2 OS=Homo sapiens GN=HDAC2 PE=1 SV=2 HDAC2_HUMAN

Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 HS90B_HUMAN

Glucose-6-phosphate isomerase OS=Homo sapiens GN=GPI PE=1 SV=4 G6PI_HUMAN

T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3 TCPZ_HUMAN

Coatamer subunit delta OS=Homo sapiens GN=ARCN1 PE=1 SV=1 COPD_HUMAN