

# ENCODE DCC Antibody Validation Document

Date of Submission

Name:

Email:

Lab

Antibody Name:

Target:

Company/  
Source:

Catalog Number, database ID, laboratory

Lot Number

Antibody  
Description:

Target  
Description:

Species Target

Species Host

Validation Method #1

Validation Method #2

Purification  
Method

Polyclonal/  
Monoclonal

Vendor URL:

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

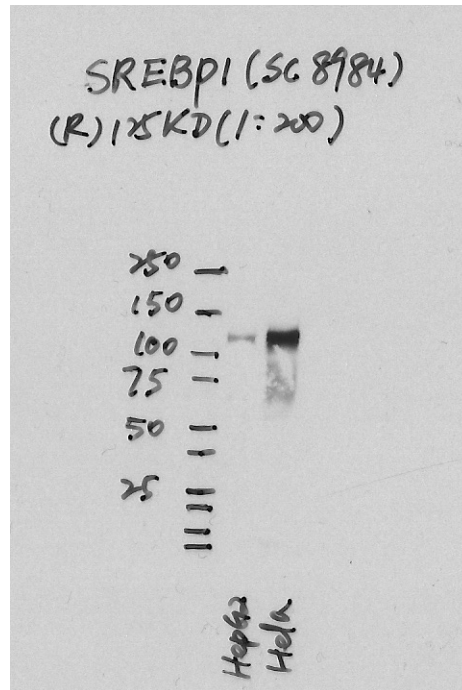
AA Position

Modification

Western blot of HeLaS3 and HepG2 nuclear extracts using the anti SREBP1 (sc-8984) detects a single high molecular weight protein of about 125kD. This band was further analyzed by IP-Mass Spec.

Validation #1  
Analysis

Insert Validation Image (click here)



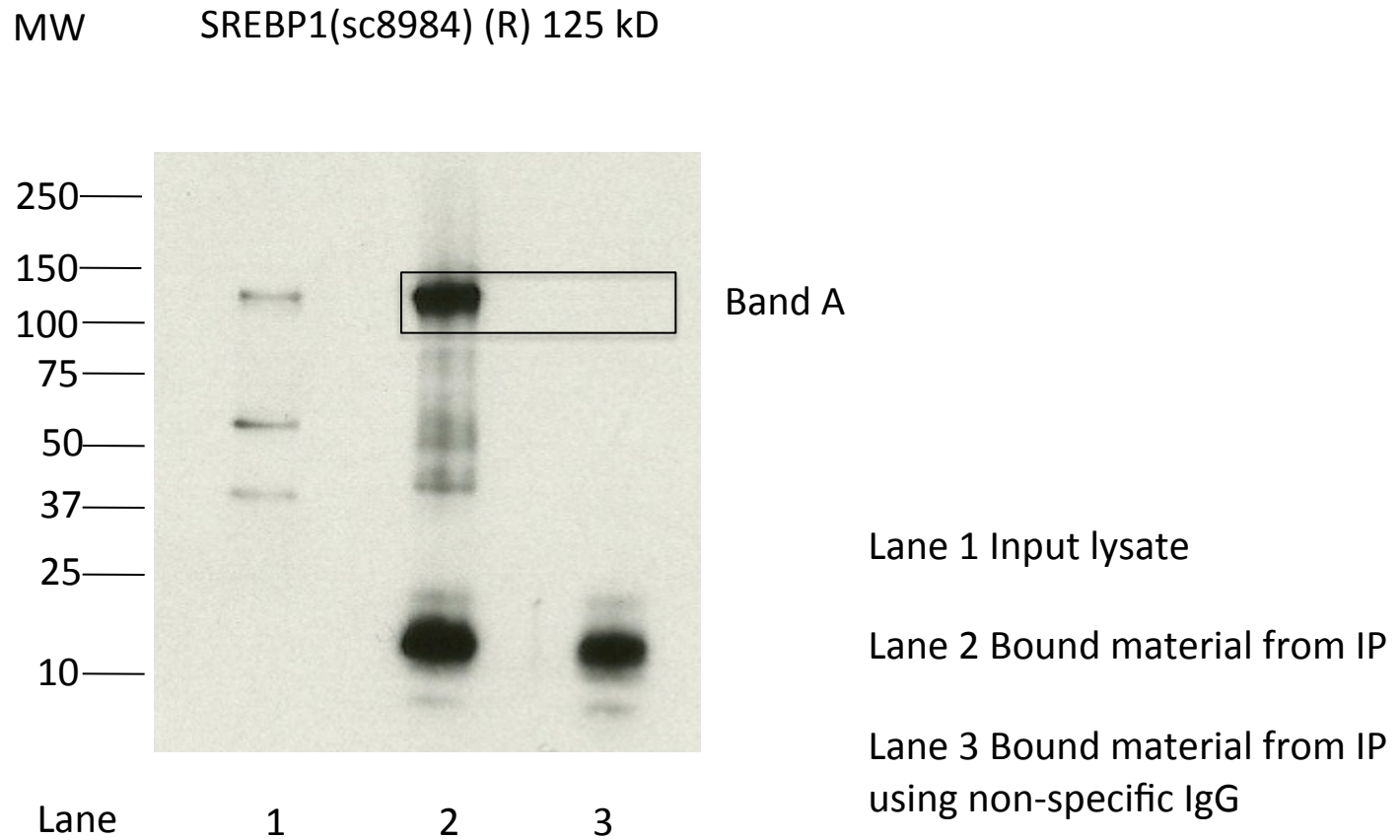
Western Blot analysis of nuclear extracts from HeLaS3 and HepG2 using anti SREBP1 antibody (sc-8984). Expected protein band 125 kD.

Immunoprecipitation of SREBP1 from HepG2 cells using sc-8984 antibody. Lane 1: input nuclear lysate. Lane 2: material immunoprecipitated with sc-8984. Lane 3: material immunoprecipitated using control IgG. Band A was excised from the gel and subjected to analysis by mass spectrometry. Briefly, protein was immunoprecipitated from HepG2 whole cell lysates using sc-8984 and the IP fraction was loaded on a 10% polyacrylamide gel (CellPAE Bis Tris Gel) and separated with an Invitrogen NUPAGE electrophoresis system. The gel was silver-stained, gel fragments corresponding to the bands indicated were excised and destained using the Silver Smap Stain for Mass Spectrometry (Perce). Then proteins were digested using the in-gel digestion method. Digested proteins were analyzed on an LTO Orbitrap (Thermo Scientific) by the nano LC-ESI-MS/MS technique. Peptides were identified by the SEQUEST algorithm and filtered with a high confidence threshold (Protein false discovery rate < 1%, 2 peptides per protein minimum). We report 27 proteins identified in band A, although 3 of these are also present in a control immunoprecipitation and are thus likely to present due to non-specific association with the matrix. Of the specifically immunoprecipitated proteins, SREBP1 is the most abundant (40 peptides). Based on these observations, this band is likely due to the presence of immunoprecipitated SREBP1 and sc-8984 meets the ENCODE standard for validation by this criterion.

Validation #2  
Analysis

Insert Validation Image (Click here)

# Immunoprecipitation assay (IP) + mass spectrometry assay



<b>Spectrum</b>	<b>Name of Protein</b>	<b>Count of peptides</b>	<b>Ratio(SREBP1/IgG Control)</b>
SREBP1 Band A	Isoform 4 of Sterol regulatory element-binding protein 1	40	NOT IN CONTROL IP
SREBP1 Band A	Oxysterol-binding protein	31	NOT IN CONTROL IP
SREBP1 Band A	Putative uncharacterized protein SFRS14	21	NOT IN CONTROL IP
SREBP1 Band A	10-formyltetrahydrofolate dehydrogenase	18	NOT IN CONTROL IP
SREBP1 Band A	Formyltetrahydrofolate dehydrogenase isoform a variant	18	NOT IN CONTROL IP
SREBP1 Band A	Isoform 1 of N-terminal kinase-like protein	18	NOT IN CONTROL IP
SREBP1 Band A	Hemoglobin subunit beta	17	NOT IN CONTROL IP
SREBP1 Band A	Isoform 5 of N-terminal kinase-like protein	17	NOT IN CONTROL IP
SREBP1 Band A	Isoform 2 of Oxysterol-binding protein-related protein 8	15	NOT IN CONTROL IP
SREBP1 Band A	interleukin enhancer-binding factor 3 isoform d	14	NOT IN CONTROL IP
SREBP1 Band A	Isoform 1 of Interleukin enhancer-binding factor 3	14	NOT IN CONTROL IP
SREBP1 Band A	Endoplasmic reticulum chaperone protein	10	0.833333333
SREBP1 Band A	HNRPU protein	9	0.529411765
SREBP1 Band A	Isoform Long of Heterogeneous nuclear ribonucleoprotein U	9	0.529411765
SREBP1 Band A	Isoform RF1/RF2 of Retrotransposon-derived protein PEG10	9	NOT IN CONTROL IP
SREBP1 Band A	Matrin-3	9	NOT IN CONTROL IP
SREBP1 Band A	Putative uncharacterized protein MATR3	9	NOT IN CONTROL IP
SREBP1 Band A	retrotransposon-derived protein PEG10 isoform 3	9	NOT IN CONTROL IP
SREBP1 Band A	Aldehyde dehydrogenase 1 family, member L1, isoform CRA_a	7	NOT IN CONTROL IP
SREBP1 Band A	Isoform 1 of Splicing factor 3B subunit 3	7	NOT IN CONTROL IP
SREBP1 Band A	Isoform 1 of Serum albumin	4	NOT IN CONTROL IP
SREBP1 Band A	FACT complex subunit SPT16	3	NOT IN CONTROL IP
SREBP1 Band A	Isoform RF1 of Retrotransposon-derived protein PEG10	3	NOT IN CONTROL IP
SREBP1 Band A	retrotransposon-derived protein PEG10 isoform 4	3	NOT IN CONTROL IP
SREBP1 Band A	Desmoplakin Ia	2	NOT IN CONTROL IP
SREBP1 Band A	Isoform DPI of Desmoplakin	2	NOT IN CONTROL IP
SREBP1 Band A	Isoform DPII of Desmoplakin	2	NOT IN CONTROL IP