

# 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:

Affinity purified rabbit polyclonal antibody raised against amino acids 455-469 (SPFLLEDDAKVKDEPDS) of human SREBP2.

Target  
Description:

SREBP-2 regulates cholesterol synthesis by activating the transcription of genes for HMG-CoA reductase and other enzymes of the cholesterol synthetic pathway. SREBP-2 is ubiquitously detected in various tissues. Under basal conditions, SREBP is bound to ER membranes as a glycosylated precursor protein. Upon cholesterol depletion, the protein is cleaved to its active form (about 50-68 kDa) and translocated into the nucleus to stimulate transcription of genes involved in the uptake and synthesis of cholesterol.

Species Target

Species Host

Validation Method #1

Validation Method #2

Purification Method

Polyclonal/  
Monoclonal

Vendor URL:

Reference (PI/  
Publication  
Information)

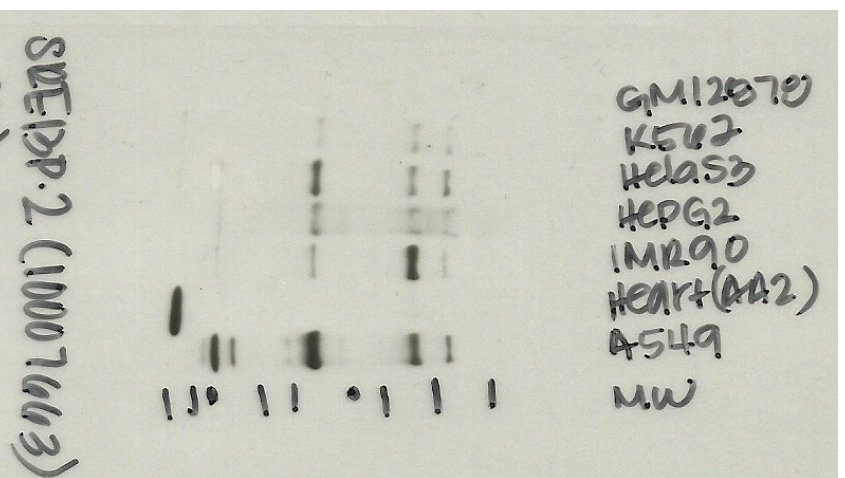
Please complete the following for antibodies to histone modifications:  
if your specifications are not listed in the drop-down box,  
please write-in the appropriate information

Histone Name  AA modified  AA Position  Modification

Western blots on nuclear lysates from cell lines GM12878, K562, HeLaS3, HepG2, IMR90, human heart tissue and A549. The antibody detects proteins ~50 kD and higher than 125kD as indicated in manufacturer product sheet. Bands in the expected size range were observed in multiple human cell types. The bands detected by anti SREBF 2 antibody (10007663) were analyzed further by IP-Mass Spec.

Validation #1  
Analysis

Insert Validation Image (click here)



Western Blot analysis of nuclear extracts from GM12878, K562, HeLaS3, HEPG2, IMR90, Human Heart, A549 using anti SREBP2 antibody from Cayman Chemicals (10007663). Expected protein band 50-68, 126 kD

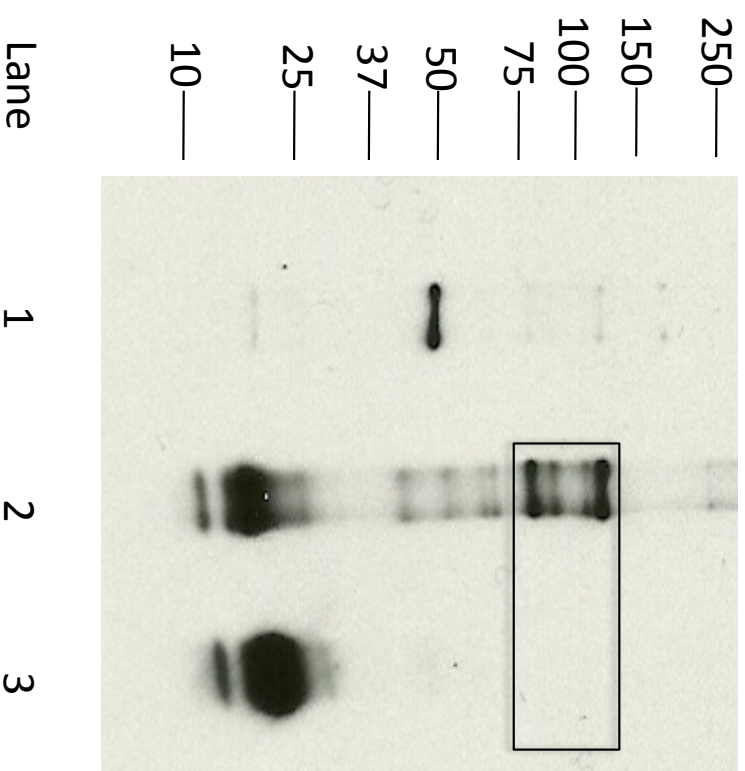
0611 Immunoprecipitation of SREBP2 from HepG2 cells using 10007663 antibody. Lane 1: input nuclear lysate. Lane 2: material immunoprecipitated with 10007663. Lane 3: material immunoprecipitated using control IgG. Bands A was excised from the gel and subjected to analysis by mass spectrometry. Briefly, protein was immunoprecipitated from HepG2 whole cell lysates using 10007663, and the I<sub>2</sub> fraction was loaded on a 10% polyacrylamide gel (NuPAGE Bis-Tris Gel) and separated with an Invitrogen NuPAGE electrophoresis system. The gel was silver-stained, gel slabs corresponding to the bands indicated were excised and destained using the SilverSAD<sup>®</sup> Stain for Mass Spectrometry (Pierce). Then proteins were digested using the in-gel digestion method. Digested proteins were analyzed on an LC-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 26 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 IP matrix. Of the specifically immunoprecipitated proteins, SREBP2 is the most abundant (185 peptides). Based on these observations, this band is likely due to the presence of immunoprecipitated SREBP2 and 10007663 meets the ENCODE standard for validation by this criterion.

Validation #2  
Analysis

Insert Validation Image (Click here)

# Immunoprecipitation assay (IP) + mass spectrometry assay

MW SREBP2(10007663) (R) 125 kD



Lane 1 Input lysate

Lane 2 Bound material from IP

Lane 3 Bound material from IP  
using non-specific IgG

<b>Spectrum</b>	<b>Name of Protein</b>	<b>Count of Peptides</b>	<b>Ratio (SREBP2/1µg Control)</b>
SREBP2 Band A	Sterol regulatory element-binding protein 2	185	NOT IN CONTROL IP
SREBP2 Band A	Isoform 1 of Myosin-9	41	NOT IN CONTROL IP
SREBP2 Band A	Isoform 1 of Myosin-10	32	NOT IN CONTROL IP
SREBP2 Band A	Endoplasmic reticulum chaperone protein	22	4.4
SREBP2 Band A	HNRPU protein	14	3.5
SREBP2 Band A	Isoform 1 of Alsln	14	NOT IN CONTROL IP
SREBP2 Band A	Isoform 5 of Interleukin enhancer-binding factor 3	14	NOT IN CONTROL IP
SREBP2 Band A	Isoform 2 of Protein LAS1 homolog	12	NOT IN CONTROL IP
SREBP2 Band A	Isoform 5 of SUN domain-containing protein 1	12	NOT IN CONTROL IP
SREBP2 Band A	Isoform Long of Spectrin beta chain, brain 1	12	NOT IN CONTROL IP
SREBP2 Band A	Isoform 5 of Fibronectin	11	NOT IN CONTROL IP
SREBP2 Band A	testis-expressed sequence 10 protein isoform 2	11	NOT IN CONTROL IP
SREBP2 Band A	ATP-dependent RNA helicase A	9	NOT IN CONTROL IP
SREBP2 Band A	Vimentin	9	NOT IN CONTROL IP
SREBP2 Band A	WD repeat and HMG-box DNA-binding protein 1	9	NOT IN CONTROL IP
SREBP2 Band A	Isoform 2 of Paternally-expressed gene 3 protein	8	NOT IN CONTROL IP
SREBP2 Band A	HEAT repeat-containing protein 1	7	NOT IN CONTROL IP
SREBP2 Band A	Leucine-rich PPR motif-containing protein, mitochondrial	7	NOT IN CONTROL IP
SREBP2 Band A	Actin, alpha skeletal muscle	6	NOT IN CONTROL IP
SREBP2 Band A	Heat shock protein HSP 90-beta	6	6
SREBP2 Band A	Isoform 3 of DNA repair protein RAD50	6	NOT IN CONTROL IP
SREBP2 Band A	alpha-actinin-1 isoform c	5	NOT IN CONTROL IP
SREBP2 Band A	Isoform 3 of Spectrin alpha chain, brain	5	NOT IN CONTROL IP
SREBP2 Band A	Heat shock protein 75 kDa, mitochondrial	4	NOT IN CONTROL IP
SREBP2 Band A	Topoisomerase II beta	4	NOT IN CONTROL IP
SREBP2 Band A	Isoform 1 of Alsln	2	NOT IN CONTROL IP