

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:

This is a rabbit polyclonal antibody generated using a synthetic peptide made to a C-terminal portion of the human DEC1 protein (between residues 350-412) as immunogen.

Target
Description:

DEC1 is a basic helix-loop-helix transcription factor that appears to play a role in cell differentiation, proliferation, apoptosis, and the circadian rhythm. DEC1 is able to promote chondrogenic and osteogenic differentiation in mesenchymal stem cells by activating transcription of bone-related genes. When it is overexpressed in cells it can suppress apoptosis by blocking caspase activation and inducing transcription

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

Validation #1
Analysis

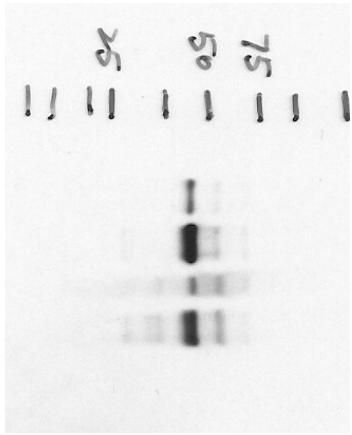
A. Western blots on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4).

B. Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody NB100-1800 against Dec1. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with NB100-1800. Lane 3: Bound material from immunoprecipitation with NB100-1800. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (18kD) that is highly enriched in the specifically immunoprecipitated fraction. Bands indicated by * in K562 immunoprecipitate are IgG heavy and light chains.

Comment: A unique band of ~49kD is detected by Western blotting with NB100-1800 in multiple human cell lines and immunoprecipitation from K562 nuclear lysate efficiently enriches a single protein of ~49kD. Based on these observations, this antibody meets this ENCODE criterion.

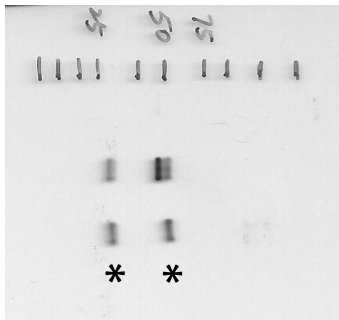
Insert Validation Image (click here)

A.



1 2 3 4

B.



1 2 3 4

Validation #2
Analysis

Immunoprecipitation of DEC1 from K562 cells using NB100-1800. Lane 1: input nuclear lysate, Lane 2: material immunoprecipitated with NB100-1800, Lane 3: material immunoprecipitated using control IgG. Bands A was excised from the gel and subject to analysis by mass spectrometry.

IP followed by mass spectrometry: Briefly, protein was immunoprecipitated from K562 whole cell lysates using NB100-1800, and the IP 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 fragments corresponding to the bands indicated were excised and destained using the SilverSNAP Stain for Mass Spectrometry (Pierce). Then proteins were trypsinized using the in-gel digestion method. Digested proteins were analyzed on an LTQ-Orbitrap (Thermo Scientific) by the nanoLC-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 12 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, DEC1 is the most abundant (42 peptides).

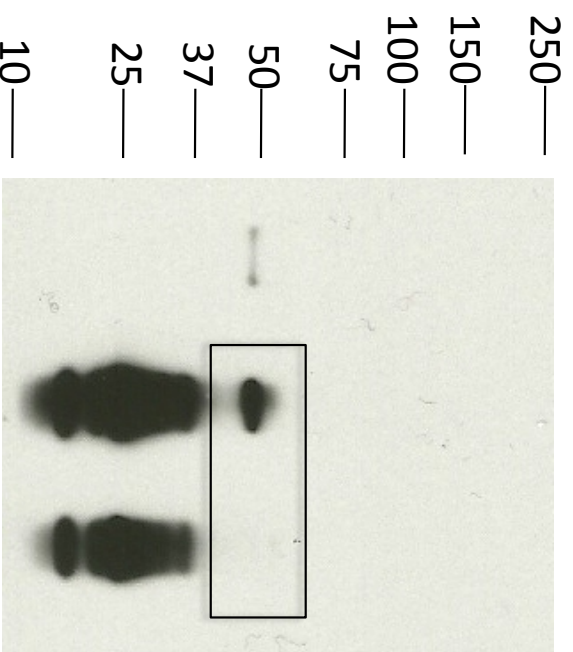
Based on these observations, this band is likely due to the presence of immunoprecipitated DEC1 and NB100-1800 meets the ENCODE standard for validation by this criterion.

Insert Validation Image (Click here)

Submit by Email

Immunoprecipitation assay (IP) + mass spectrometry assay

MW DEC-1(NB100-1800) (R) 51 kD



Band A

Lane 1 Input lysate

Lane 2 Bound material from IP

Lane 3 Bound material from IP
using non-specific IgG

Lane 1 2 3

Spectrum	Name of Protein	Count of peptides	Ratio(DEC1/IgG Control)
DEC1_Band A	Class E basic helix-loop-helix protein 40	42	NOT IN CONTROL IP
DEC1_Band A	CDNA FLJ56903, highly similar to Tubulin beta-7 chain	15	1.153846154
DEC1_Band A	ATP synthase subunit alpha, mitochondrial	10	2
DEC1_Band A	Dermcidin	9	NOT IN CONTROL IP
DEC1_Band A	CDNA FLJ53012, highly similar to Tubulin beta-7 chain	7	1.4
DEC1_Band A	Desmoglein-1	6	NOT IN CONTROL IP
DEC1_Band A	Isoform 1 of RuvB-like 1	6	NOT IN CONTROL IP
DEC1_Band A	Interleukin enhancer-binding factor 2	5	NOT IN CONTROL IP
	CDNA FLJ55805, highly similar to Keratin, type II cytoskeletal 4	3	NOT IN CONTROL IP
DEC1_Band A	RuvB-like 2	3	NOT IN CONTROL IP
DEC1_Band A	Putative uncharacterized protein HNRNPAB	2	NOT IN CONTROL IP
DEC1_Band A	Isoform 2 of Mucin-19	1	NOT IN CONTROL IP