

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:

CDP (C-20) is an affinity purified goat polyclonal antibody raised against a peptide mapping at the C-terminus of CDP of mouse origin.

Target
Description:

CDP (for CCAAT displacement protein) was identified as a repressor for transcription of developmentally regulated genes. It is a homeodomain protein that appears to compete with transcriptional activating proteins for binding to the promoter regions of various genes. CDP contains three cut repeats which function as DNA binding domains. It has been demonstrated that cut repeat domains have the capacity to bind to DNA in conjunction with or independently of homeodomain DNA binding. CDP has been shown to be the DNA-binding subunit of the HNF-1 complex, which contains cyclin A, Cdc2 and an Rb-related protein in addition to CDP. Histone expression is required for the transition to S phase in the cell cycle. The HNF-1 complex regulates the transcription of Histone H4, H3 and H1 genes, allowing cells to progress from G1 to S phase.

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

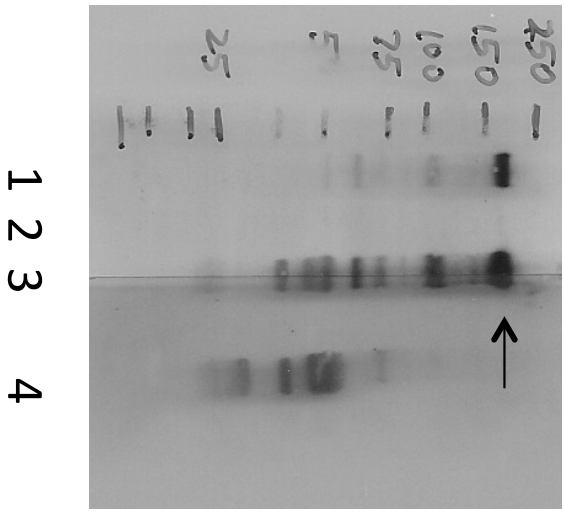
Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody SC6327 against CDP. Lane 1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with SC6327. Lane 3: Bound material from immunoprecipitation with SC6327. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (~180 kD) that is highly enriched in the specifically immunoprecipitated fraction.

Comment: Immunoprecipitation from K562 nuclear lysate enriches a protein of ~180kD. There are some low molecular weight bands which could be possible degradation products. The other non-specific bands are also present in control IP. Based on these observations, this antibody meets this ENCODE criterion.

Validation #1
Analysis

Insert Validation Image (click here)

A.



Immunoprecipitation of CDP from K562 cells using SC6327. Lane 1: input nuclear lysate, Lane 2: material immunoprecipitated with SC6327, 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 SC6327 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 fractions corresponding to the bands indicated were excised and destained using the Silver Shield Stain for Mass Spectrometry (Perca). Then proteins were digested using the in-gel digestion method. Digested proteins were analyzed on an LFC 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 29 proteins identified in band A. Of the specifically immunoprecipitated proteins, CDP is the most abundant (373 peptides).

Based on these observations, this band is likely due to the presence of immunoprecipitated CDP and SC6327 meets the ENCODE standard for validation by this criterion.

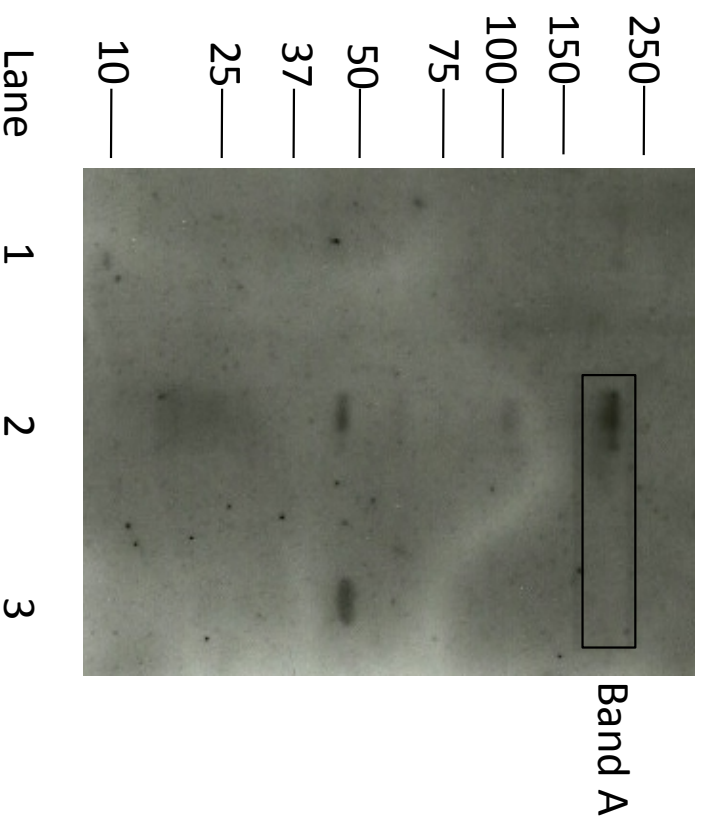
Validation #2
Analysis

Insert Validation Image (Click here)

Submit by Email

Immunoprecipitation assay (IP) + mass spectrometry assay

MW CDP (sc6327x) (G) 180 kD



Lane 1 Input lysate

Lane 2 Bound material from IP

Lane 3 Bound material from IP using non-specific IgG

Spectrum	Name of Protein	Count of Peptides	Ratio(CDP/IgG Control)
CDP_Band A	Isoform 6 of Homeobox protein cut-like 1 (CDP)	373	NOT IN CONTROL IP
CDP_Band A	Protein CASP	37	NOT IN CONTROL IP
CDP_Band A	Elongation factor 1-alpha	15	NOT IN CONTROL IP
CDP_Band A	Histone H1.2	8	NOT IN CONTROL IP
CDP_Band A	Histone H1.3	8	NOT IN CONTROL IP
CDP_Band A	Protein S100-A9	8	NOT IN CONTROL IP
CDP_Band A	X-ray repair cross-complementing protein 5	8	NOT IN CONTROL IP
CDP_Band A	Heat shock protein HSP 90-beta	7	1.4
CDP_Band A	Histone H2A.x	7	NOT IN CONTROL IP
CDP_Band A	Histone H1.4	5	NOT IN CONTROL IP
CDP_Band A	Interleukin enhancer-binding factor 3 isoform d	5	2.5
CDP_Band A	Nucleolar protein 11	5	NOT IN CONTROL IP
CDP_Band A	Putative elongation factor 1-alpha-like 3	5	NOT IN CONTROL IP
CDP_Band A	Tubulin, beta	5	NOT IN CONTROL IP
CDP_Band A	Glyceraldehyde-3-phosphate dehydrogenase	4	NOT IN CONTROL IP
CDP_Band A	Lamin A/C	4	3.5
CDP_Band A	ALB protein	3	NOT IN CONTROL IP
CDP_Band A	DNA topoisomerase 2	3	NOT IN CONTROL IP
CDP_Band A	Heterogeneous nuclear ribonucleoprotein A1-like 2	3	NOT IN CONTROL IP
CDP_Band A	Putative heterogeneous nuclear ribonucleoprotein A1-like 3	3	NOT IN CONTROL IP
CDP_Band A	Similar to Heterogeneous nuclear ribonucleoprotein A1	3	NOT IN CONTROL IP
CDP_Band A	CDNA FLJ77784	2	NOT IN CONTROL IP
CDP_Band A	NOP56 protein (Fragment)	2	NOT IN CONTROL IP
CDP_Band A	Nucleolar protein 56	2	NOT IN CONTROL IP
CDP_Band A	Nucleolar protein 5A (Fragment)	2	NOT IN CONTROL IP
CDP_Band A	Progerin	2	NOT IN CONTROL IP
CDP_Band A	Rhabdomyosarcoma antigen MU-RMS-40.12	2	NOT IN CONTROL IP
CDP_Band A	Probable ATP-dependent RNA helicase DDX5	1	NOT IN CONTROL IP
CDP_Band A	Putative annexin A2-like protein	1	NOT IN CONTROL IP