

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 as immunogen a region that maps between residues 1700 and 1750 of human host cell factor C1.

Target
Description:

This gene is a member of the host cell factor family and encodes a protein with five Kelch repeats, a fibronectin-like motif, and six HCF repeats, each of which contains a highly specific cleavage signal. This nuclear coactivator is proteolytically cleaved at one of the six possible sites, resulting in the creation of an N-terminal chain and the corresponding C-terminal chain. The final form of this protein consists of

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

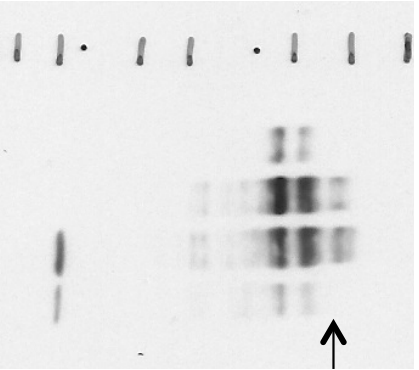
A. Western blots on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4).

B. Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody NB100-68209 against HCFC1. Lane 1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with NB100-68209. Lane 3: Bound material from immunoprecipitation with NB100-68209. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (208 kD) that is enriched in the specifically immunoprecipitated fraction. Smaller band detected in the IP is possibly degradation product as indicated by the Mass Spec analysis. Band indicated by * in K562 immunoprecipitate is IgG light chains.

Comment: A high molecular weight band (~208 kD) is detected by Western blotting with NB100-68209 in multiple human cell lines. Immunoprecipitation from K562 nuclear lysate enriches a protein of ~208 KD. Based on these observations, this antibody meets this ENCODE criterion.

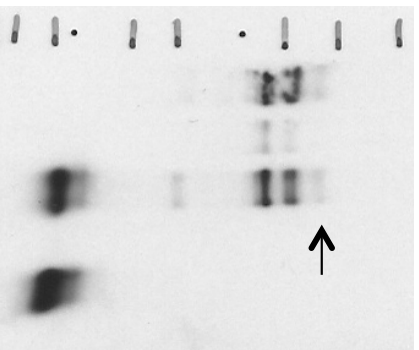
Insert Validation Image (click here)

A.



1
2
3
4

B.



1
2
3
4

*

Immunoprecipitation of HCFC1 from K562 cells using NB100-68209. Lane 1: input nuclear lysate, Lane 2: material immunoprecipitated with NB100-68209, Lane 3: material immunoprecipitated using control IgG. Bands A and B were 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 NB100-68209, 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 29 different proteins identified in band A and B. Of the specifically immunoprecipitated proteins, HCFC1 is the most abundant protein in both band A as well as band B. Based on these observations, this band is likely due to the presence of immunoprecipitated HCFC1 and NB100-68209 meets the ENCODE standard for validation by this criterion.

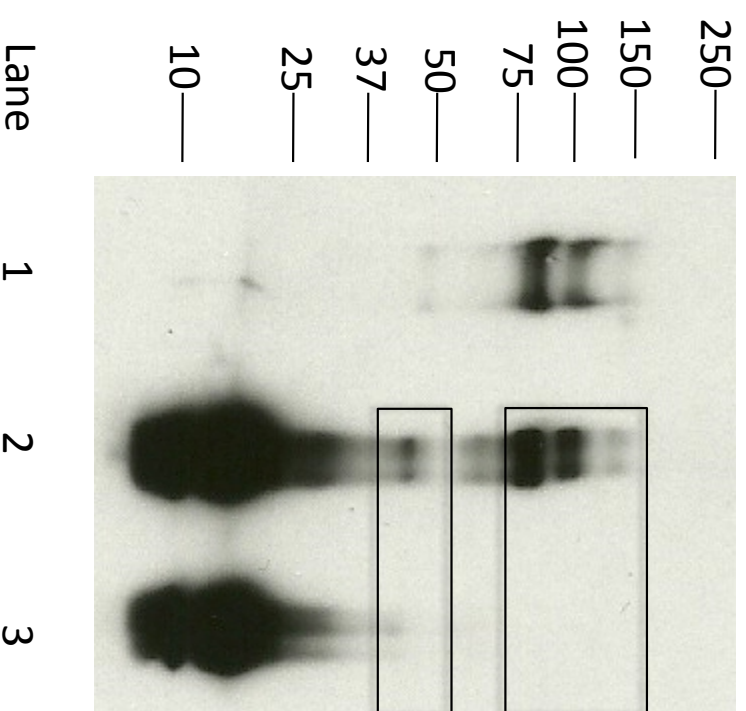
Validation #2
Analysis

Insert Validation Image (Click here)

Submit by Email

Immunoprecipitation assay (IP) + mass spectrometry assay

MW HCFC1 (NB100-68209) (R) 208 kD



Band A

Band B

Lane 1 Input lysate

Lane 2 Bound material from IP

Lane 3 Bound material from IP
using non-specific IgG

| Spectra | Name of Protein | Count of Peptides | Ratio (HCFC1/IgG Control) |
|--------------|--|-------------------|---------------------------|
| HCFC1 Band A | Putative uncharacterized protein HCFC1 | 302 | NOT IN CONTROL IP |
| HCFC1 Band A | CDNA FLU55383 | 11 | NOT IN CONTROL IP |
| HCFC1 Band A | Histone-lysine N-methyltransferase SETD1A | 11 | NOT IN CONTROL IP |
| HCFC1 Band A | Isoform 1 of MLL1/MLL complex subunit KIAA1267 | 10 | NOT IN CONTROL IP |
| HCFC1 Band A | Horrerin | 6 | NOT IN CONTROL IP |
| HCFC1 Band A | Isoform 2 of Kinesin-like protein KIF2C | 5 | NOT IN CONTROL IP |
| HCFC1 Band A | PHD finger protein 20-like protein 1 isoform 1 | 3 | NOT IN CONTROL IP |
| HCFC1 Band A | similar to protein kinase, DNA-activated, catalytic polypeptide | 2 | NOT IN CONTROL IP |
| HCFC1 Band A | DNA replication licensing factor MCM7 isoform 2 | 1 | NOT IN CONTROL IP |
| HCFC1 Band A | Isoform 1 of PHD finger protein 20 | 1 | NOT IN CONTROL IP |
| HCFC1 Band B | Putative uncharacterized protein HCFC1 | 89 | NOT IN CONTROL IP |
| HCFC1 Band B | Isoform 4 of Heterogeneous nuclear ribonucleoproteins C1/C2 | 21 | 2.333333333 |
| HCFC1 Band B | WD repeat-containing protein 5 | 18 | NOT IN CONTROL IP |
| HCFC1 Band B | ATP synthase subunit alpha | 14 | NOT IN CONTROL IP |
| HCFC1 Band B | ATP synthase subunit beta, mitochondrial | 14 | NOT IN CONTROL IP |
| HCFC1 Band B | Serine/threonine-protein phosphatase PP1-alpha catalytic subunit | 12 | NOT IN CONTROL IP |
| HCFC1 Band B | Isoform 3 of Microspherule protein 1 | 9 | NOT IN CONTROL IP |
| HCFC1 Band B | Tubulin, beta | 9 | NOT IN CONTROL IP |
| HCFC1 Band B | CDNA FLU56579, highly similar to Prohibitin-2 | 6 | NOT IN CONTROL IP |
| HCFC1 Band B | Elongation factor 1-alpha | 6 | NOT IN CONTROL IP |
| HCFC1 Band B | Isoform 1 of MLL1/MLL complex subunit KIAA1267 | 6 | NOT IN CONTROL IP |
| HCFC1 Band B | PHD finger protein 20-like protein 1 isoform 1 | 6 | NOT IN CONTROL IP |
| HCFC1 Band B | RNA binding protein, autoantigenic | 6 | NOT IN CONTROL IP |
| HCFC1 Band B | CDNA FLU54408, highly similar to Heat shock 70 kDa protein 1 | 3 | NOT IN CONTROL IP |
| HCFC1 Band B | Heat shock 70 kDa protein 1A/1B | 3 | NOT IN CONTROL IP |
| HCFC1 Band B | HSPA5 protein | 3 | NOT IN CONTROL IP |
| HCFC1 Band B | Isoform 1 of PHD finger protein 20 | 3 | NOT IN CONTROL IP |
| HCFC1 Band B | Isoform 1 of Probable histone acetyltransferase MVST1 | 2 | NOT IN CONTROL IP |
| HCFC1 Band B | CDNA FLU55383 | 1 | NOT IN CONTROL IP |