

# 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 Zinc finger protein 384 recombinant protein epitope signature tag (PrEST) as an immunogen.

Target  
Description:

This gene encodes a C2H2-type zinc finger protein, which may function as a transcription factor. This gene also contains long CAG trinucleotide repeats that encode consecutive glutamine residues. The protein appears to bind and regulate the promoters of the extracellular matrix genes MMP1, MMP3, MMP7 and COL1A1. Studies in mouse suggest that nuclear matrix transcription factors (NP/NMP4) may be part of a

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

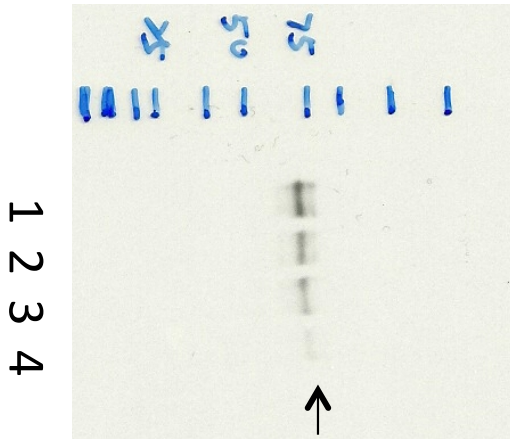
A. Western blots on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4).

B. Immunoprecipitation was performed on nuclear lysates from HeLaS3 cells using antibody HPA004051 against ZNF384. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with HPA004051. Lane 3: Bound material from immunoprecipitation with HPA004051. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (~63 kD) that is efficiently enriched in the specifically immunoprecipitated fraction. Band indicated by \* in HeLaS3 immunoprecipitate is IgG light chains.

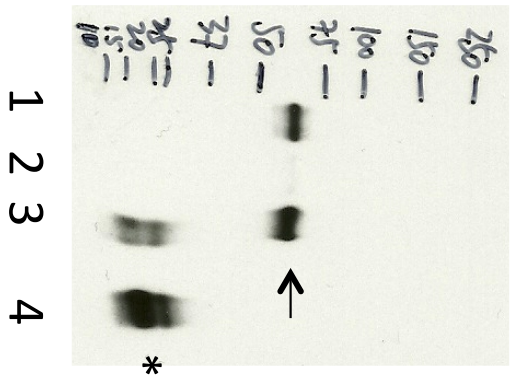
Comment: A single band of the correct weight band (~208 kD) is detected by Western blotting with HPA004051 in multiple human cell lines. Immunoprecipitation from HeLaS3 nuclear lysate specifically enriches a single protein of ~63KD. Based on these observations, this antibody meets this ENCODE criterion.

Insert Validation Image (click here)

A.



B.



Validation #2  
Analysis

Immunoprecipitation of ZNF384 from K562 cells using HPA004051. Lane 1: input nuclear lysate, Lane 2: material immunoprecipitated with HPA004051, Lane 3: material immunoprecipitated using control IgG. Bands A was excised from the gel and subject to analysis by mass spectrometry.

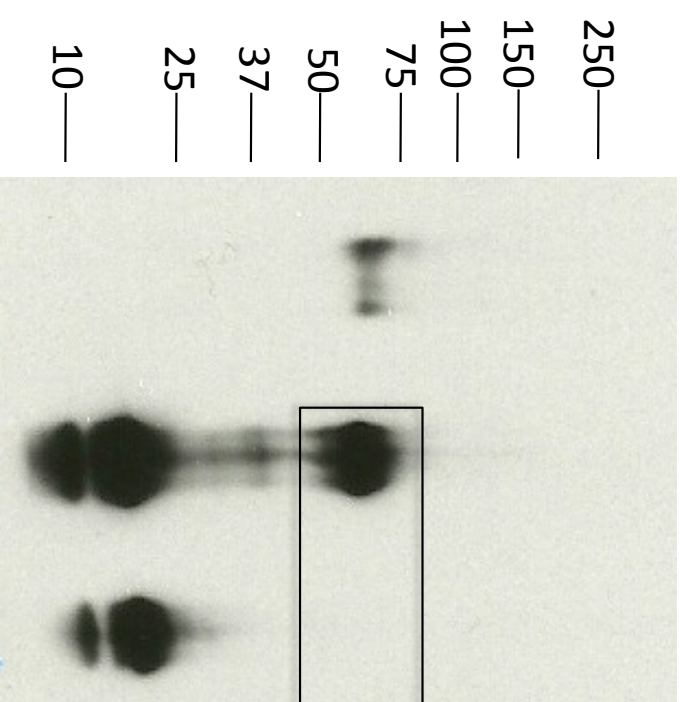
IP followed by mass spectrometry: Briefly, protein was immunoprecipitated from K562 whole cell lysates using NB100-68209, and the IP fraction was loaded on a 10% polyacrylamide gel (NuPAGE Bis-Tris Gel) and separated with an Invitrogen NuPAGE electrophoresis system. The gel was silver-stained, gel fragments 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 20 different proteins identified in band A, of this 9 were also detected in control IP indicating non specific enrichment during immunoprecipitation. Of the specifically immunoprecipitated proteins, ZNF384 is the most abundant protein in both band A . Based on these observations, this band is likely due to the presence of immunoprecipitated ZNF384 and HPA004051 meets the ENCODE standard for validation by this criterion.

Insert Validation Image (Click here)

Submit by Email

# Immunoprecipitation assay (IP) + mass spectrometry assay

MW ZNF384 (HPA004051) (R) 63 kD



Lane 1 2 3

Band A

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(ZNF384/IgG Control)</b> |
|-----------------|---|--------------------------|----------------------------------|
| ZNF384 Band A   | Isoform 2 of Zinc finger protein 384                                | 107                      | NOT IN CONTROL IP                |
| ZNF384 Band A   | Isoform 1 of Heat shock cognate 71 kDa protein                      | 32                       | 3.555555556                      |
| ZNF384 Band A   | Putative uncharacterized protein LMNA                               | 29                       | 14.5                             |
| ZNF384 Band A   | HSPA5 protein   | 25                       | 25                               |
| ZNF384 Band A   | Stress-70 protein, mitochondrial                                    | 24                       | 1.043478261                      |
| ZNF384 Band A   | DNA replication licensing factor MCM7 isoform 2                     | 23                       | NOT IN CONTROL IP                |
| ZNF384 Band A   | Lamin-B1  | 20                       | 4                                |
| ZNF384 Band A   | Isoform 2 of Heterogeneous nuclear ribonucleoprotein M              | 12                       | 12                               |
| ZNF384 Band A   | Calcium-binding mitochondrial carrier protein Aralar2               | 10                       | NOT IN CONTROL IP                |
| ZNF384 Band A   | Isoform 2 of Sarcoplasmic/endoplasmic reticulum calcium ATPase 2    | 9                        | NOT IN CONTROL IP                |
| ZNF384 Band A   | Nuclear pore complex protein Nup85                                  | 9                        | NOT IN CONTROL IP                |
| ZNF384 Band A   | Nucleolar protein 56  | 8                        | 1.333333333                      |
| ZNF384 Band A   | Isoform 2 of Sentrin-specific protease 1                            | 7                        | NOT IN CONTROL IP                |
| ZNF384 Band A   | cDNA FLJ58756, highly similar to Nuclear pore complex protein Nup93 | 6                        | NOT IN CONTROL IP                |
| ZNF384 Band A   | Isoform 1 of RNA-binding protein 14                                 | 6                        | 1.5                              |
| ZNF384 Band A   | T-complex protein 1 subunit alpha                                   | 5                        | NOT IN CONTROL IP                |
| ZNF384 Band A   | Isoform 1 of DNA-dependent protein kinase catalytic subunit         | 4                        | NOT IN CONTROL IP                |
| ZNF384 Band A   | Isoform 2 of ATPase family AAA domain-containing protein 3A         | 4                        | 0.571428571                      |
| ZNF384 Band A   | ATP-dependent RNA helicase A  | 3                        | 0.75                             |
| ZNF384 Band A   | Isoform 2 of Transcription factor Sp3                               | 3                        | NOT IN CONTROL IP                |