

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 an epitope that maps to a region between residue 250 and 300 of human zinc finger CCCH-type containing 11A.

Target
Description:

ZC3H11A is a C3H1-type zinc finger protein. ZC3H11A has been identified as a protein that is phosphorylated upon DNA damage by ATM or ATR. The function of ZC3H11A remains uncharacterized.

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

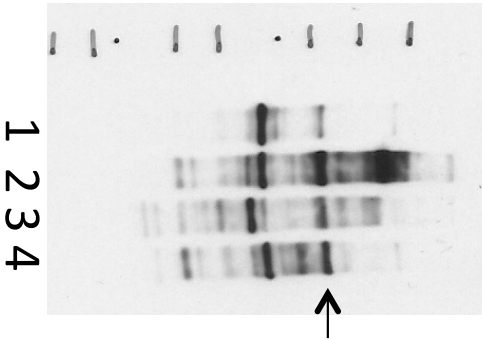
A. Western blots on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4).

B. Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody NB100-74650 against ZC3H11A. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with NB100-74650. Lane 3: Bound material from immunoprecipitation with NB100-74650. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (89kD) that is highly enriched in the specifically immunoprecipitated fraction. Bands indicated by * in K562 immunoprecipitate is IgG light chains.

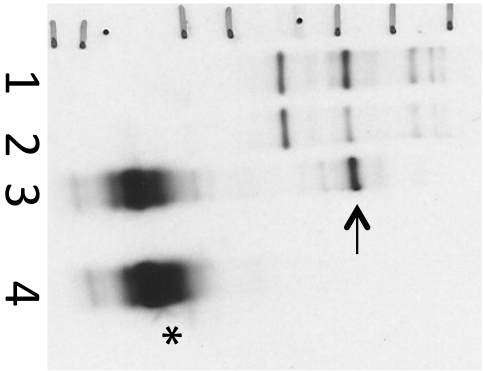
Comment: A band of ~89kD is detected by Western blotting with NB100-74650 in multiple human cell lines. Immunoprecipitation from K562 nuclear lysate efficiently enriches a single protein of ~89kD. Based on these observations, this antibody meets this ENCODE criterion.

Insert Validation Image (click here)

A.



B.



Validation #2
Analysis

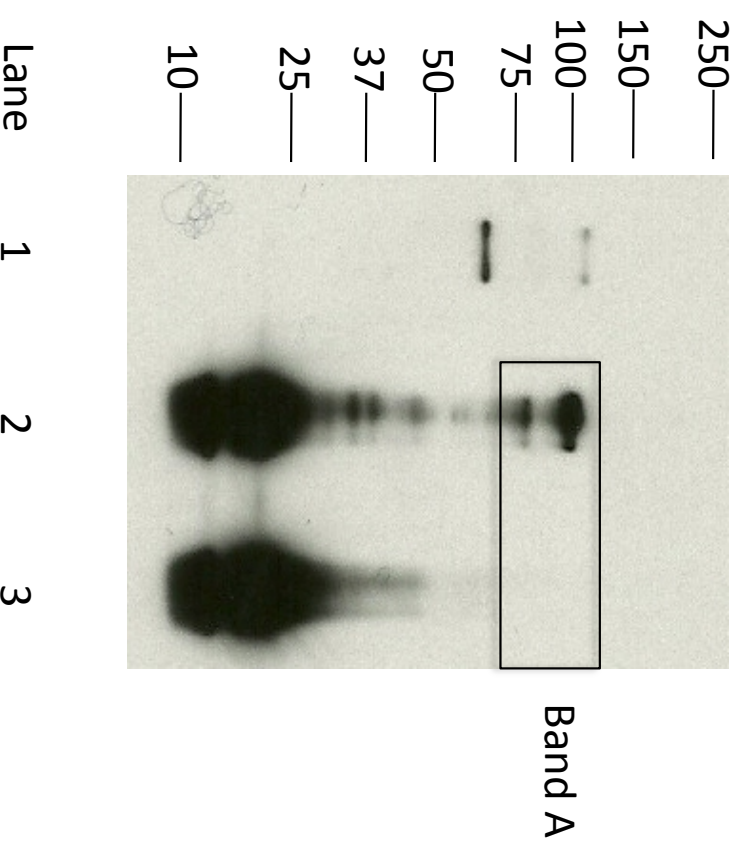
Immunoprecipitation of ZC3H11A from K562 cells using NB100-74650. Lane 1: input nuclear lysate, Lane 2: material immunoprecipitated with NB100-74650, Lane 3: material immunoprecipitated using control IgG. Bands A was excised from the gel and subject to analysis by mass spectrometry. IP followed by mass spectrometry: Briefly, protein was immunoprecipitated from K562 whole cell lysates using NB100-74650, 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 71 different proteins identified in band A. Of the specifically immunoprecipitated proteins, ZC3H11A is the most abundant (242 peptides). Based on these observations, this band is likely due to the presence of immunoprecipitated ZC3H11A and NB100-74650 meets the ENCODE standard for validation by this criterion.

Insert Validation Image (Click here)

Submit by Email

Immunoprecipitation assay (IP) + mass spectrometry assay

MW ZC3H11A(NB100-74650) (R) 117 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(ZC3H11A/IgG Control)
ZC3H11A_Band A	Zinc finger CCH domain-containing protein 11A	242	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform A of Lamin-A/C	78	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 5 of Interleukin enhancer-binding factor 3	38	NOT IN CONTROL IP
ZC3H11A_Band A	Stress-70 protein, mitochondrial	38	NOT IN CONTROL IP
ZC3H11A_Band A	ATP-dependent RNA helicase A	34	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 3 of Peroxisomal targeting signal 1 receptor	34	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 2 of Heterogeneous nuclear ribonucleoprotein M	32	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 1 of Heterogeneous nuclear ribonucleoprotein R	31	NOT IN CONTROL IP
ZC3H11A_Band A	cDNA FLJ59357, highly similar to Probable ATP-dependent RNA helicase DDX5	29	NOT IN CONTROL IP
ZC3H11A_Band A	E3 ubiquitin/ISG15 ligase TRIM25	28	NOT IN CONTROL IP
ZC3H11A_Band A	insulin-like growth factor 2 mRNA-binding protein 1 isoform 2	26	NOT IN CONTROL IP
ZC3H11A_Band A	Nucleolar protein 56	25	NOT IN CONTROL IP
ZC3H11A_Band A	THO complex subunit 5 homolog	25	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 2 of Polyadenylate-binding protein 1	22	NOT IN CONTROL IP
ZC3H11A_Band A	Lamin-B1	18	NOT IN CONTROL IP
ZC3H11A_Band A	cDNA FLJ58652, highly similar to Probable ATP-dependent RNA helicase DDX17	17	NOT IN CONTROL IP
ZC3H11A_Band A	HSPA5 protein	16	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 1 of RNA-binding protein 14	16	NOT IN CONTROL IP
ZC3H11A_Band A	cDNA FLJ54020, highly similar to Heterogeneous nuclear ribonucleoprotein U	15	NOT IN CONTROL IP
ZC3H11A_Band A	Nucleolar GTP-binding protein 1	15	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 1 of Insulin-like growth factor 2 mRNA-binding protein 3	13	NOT IN CONTROL IP
ZC3H11A_Band A	X-ray repair complementing defective repair in Chinese hamster cells 6	13	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform DPII of Desmoplakin	12	NOT IN CONTROL IP
ZC3H11A_Band A	Poly(A) binding protein, cytoplasmic 4 (Inducible form), isoform CRA_e	12	NOT IN CONTROL IP
ZC3H11A_Band A	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	12	NOT IN CONTROL IP
ZC3H11A_Band A	SYNCRIP protein (Fragment)	12	NOT IN CONTROL IP
ZC3H11A_Band A	WD repeat-containing protein 46	12	NOT IN CONTROL IP
ZC3H11A_Band A	cDNA FLJ54408, highly similar to Heat shock 70 kDa protein 1	11	NOT IN CONTROL IP
ZC3H11A_Band A	Crooked neck pre-mRNA splicing factor-like 1	11	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 1 of Nucleolar RNA helicase 2	11	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 2 of Protein LAS1 homolog	11	NOT IN CONTROL IP
ZC3H11A_Band A	Nucleolar protein 11	11	NOT IN CONTROL IP
ZC3H11A_Band A	Pre-mRNA-processing factor 6	11	NOT IN CONTROL IP
ZC3H11A_Band A	Splicing factor 3A subunit 1	11	NOT IN CONTROL IP
ZC3H11A_Band A	heterogeneous nuclear ribonucleoprotein L isoform b	10	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform Short of TATA-binding protein-associated factor 2N	9	NOT IN CONTROL IP
ZC3H11A_Band A	Seipin	9	NOT IN CONTROL IP
ZC3H11A_Band A	Calcium-binding mitochondrial carrier protein Aralar2	7	NOT IN CONTROL IP
ZC3H11A_Band A	cDNA FLJ51295, highly similar to Cell division cycle 5-like protein	7	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 1 of Splicing factor 3B subunit 3	7	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 2 of Putative ribosomal RNA methyltransferase NOP2	7	NOT IN CONTROL IP
ZC3H11A_Band A	Nuclear pore complex protein Nup205	7	NOT IN CONTROL IP
ZC3H11A_Band A	ATP-dependent RNA helicase DDX18	6	NOT IN CONTROL IP
ZC3H11A_Band A	Desmoglein-1	6	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 7 of Zinc finger CCH domain-containing protein 14	6	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform UBF2 of Nucleolar transcription factor 1	6	NOT IN CONTROL IP
ZC3H11A_Band A	THO complex subunit 2	6	NOT IN CONTROL IP
ZC3H11A_Band A	WD repeat-containing protein 36	6	NOT IN CONTROL IP
ZC3H11A_Band A	Heat shock protein HSP 90-beta	5	NOT IN CONTROL IP
ZC3H11A_Band A	Pre-mRNA-splicing factor SYF1	5	NOT IN CONTROL IP
ZC3H11A_Band A	X-ray repair cross-complementing protein 5	5	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 1 of Heat shock protein HSP 90-alpha	4	NOT IN CONTROL IP
ZC3H11A_Band A	Junction plakoglobin	4	2
ZC3H11A_Band A	Scaffold attachment factor B1	4	NOT IN CONTROL IP
ZC3H11A_Band A	WD repeat-containing protein 43	4	NOT IN CONTROL IP
ZC3H11A_Band A	cDNA FLJ45706 fis, clone FEBRA2028457, highly similar to Nucleolin	3	NOT IN CONTROL IP
ZC3H11A_Band A	DNA topoisomerase 1	3	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 1 of THO complex subunit 1	3	NOT IN CONTROL IP
ZC3H11A_Band A	Probable ATP-dependent RNA helicase DDX27	3	NOT IN CONTROL IP
ZC3H11A_Band A	DNA replication licensing factor MCM7 isoform 2	2	NOT IN CONTROL IP
ZC3H11A_Band A	DNA topoisomerase 2	2	NOT IN CONTROL IP
ZC3H11A_Band A	FACT complex subunit SPT16	2	NOT IN CONTROL IP
ZC3H11A_Band A	Histone H2A.V	2	NOT IN CONTROL IP
ZC3H11A_Band A	Isoform 3 of Zinc finger MYM-type protein 4	2	NOT IN CONTROL IP
ZC3H11A_Band A	N-acetyltransferase 10	2	NOT IN CONTROL IP
ZC3H11A_Band A	apoptotic chromatin condensation inducer in the nucleus isoform 3	1	NOT IN CONTROL IP
ZC3H11A_Band A	Histone H2B type 1-L	1	NOT IN CONTROL IP
ZC3H11A_Band A	Histone H3.2	1	NOT IN CONTROL IP
ZC3H11A_Band A	Hornerin	1	0.25
ZC3H11A_Band A	Splicing factor 3B subunit 1	1	NOT IN CONTROL IP
ZC3H11A_Band A	Thyroid hormone receptor-associated protein 3	1	NOT IN CONTROL IP