ENCODE DCC Antibody Validation Document

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Lab:

Antibody Name: ZMIZ1 (ab65767)

Target: ZMIZ1 (Entrez Gene 57178)

Company/ Source: Abcam

Catalog Number, database ID, laboratory: Ab65767

Lot Number: GR30927-1

Antibody Description: This is a rabbit polyclonal antibody generated using a synthetic peptide conjugated to KLH derived from within residues 200 - 300 of Human Zinc finger MIZ domain-containing protein 1

Target Description: Zinc finger MIZ domain-containing protein 1that has been shown to increases ligand-dependent transcriptional activity of AR and promotes AR sumoylation. The stimulation of AR activity is dependent upon sumoylation.

Species Target: Human

Species Host: Rabbit

Validation Method #1: Immunoprecipitation

Validation Method #2: IP-Mass Spec

Purification Method: Affinity

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://www.abcam.com/Zinc-finger-MIZ-do

Reference (PI/ Publication Information):

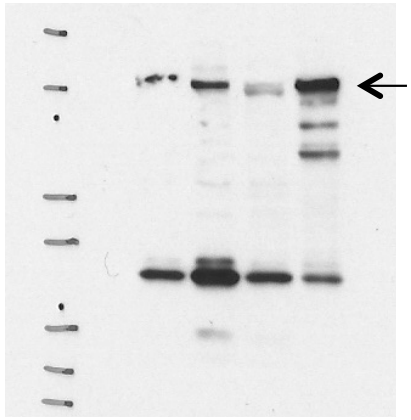
Please complete the following for antibodies to histone modifications:
if your specifications are not listed in the drop-down box, please write-in the appropriate information

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 ab65767 against ZMIZ1. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with ab65767. Lane 3: Bound material from immunoprecipitation with ab65767. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (115kD) that is enriched in the specifically immunoprecipitated fraction. Smaller bands could be possibly degradation products of ZMIZ1 protein. Band indicated by * in K562 immunoprecipitate is IgG light chains. Comment: A band of ~115 kD is detected by Western blotting with ab65767 in multiple human cell lines. Immunoprecipitation from K562 nuclear lysate enriches a protein of ~115KD. Based on these observations, this antibody meets this ENCODE criterion.

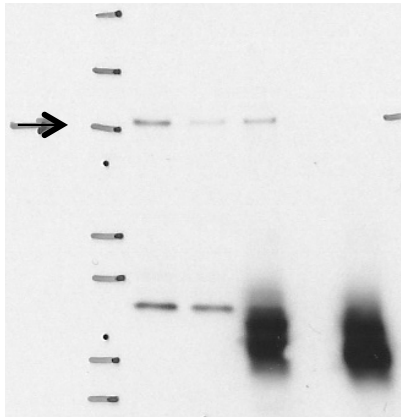
Insert Validation Image (click here)

A.



1 2 3 4

B.



1 2 3 4

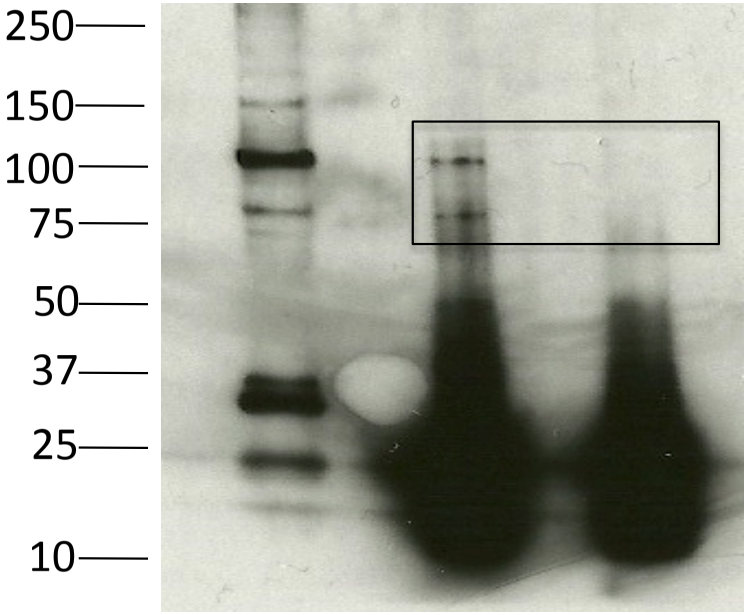
Validation #2 Analysis	Immunoprecipitation of ZMIZ1 from K562 cells using ab65767. Lane 1: input nuclear lysate, Lane 2: material immunoprecipitated with ab65767, Lane 3: material immunoprecipitated using control IgG. Bands A was excised from the gel and subject to analysis by mass spectrometry. This antibody was raised against an immunogen that is predicted to cross react with both isoform 1 (115 kDa) and isoform 2 (107 kDa) of ZMIZ1. The bands we observe at ~115 kDa and ~105 kDa could possibly correspond to teh two isoforms of ZMIZ1. IP followed by mass spectrometry: Briefly, protein was immunoprecipitated from K562 whole cell lysates using ab65767, 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 22 different proteins identified in band A, of which 9 were detected in the control IgG IP too, indicating a non specific enrichment of these proteons during immunoprecipitation. 3 out of the top 5 hits were enriched in both the ab65767 as well as control IgG IP. Of the specifically immunoprecipitated proteins, ZMIZ1 is the most abundant protein. Based on these observations, this band is likely due to the presence of immunoprecipitated ZMIZ1 and ab65767 meets the ENCODE standard for validation by this criterion.

Insert Validation Image (Click here)

Submit by Email

Immunoprecipitation assay (IP) + mass spectrometry assay

MW ZMIZ1 (ab65767)) (R) 115 kD



Spectrum	Name of protein	Count of peptides	Ratio (ZMIZ1/IgG Control)
ZMIZ1 Band A	Isoform 5 of Interleukin enhancer-binding factor 3	33	11
ZMIZ1 Band A	Isoform 1 of Heat shock cognate 71 kDa protein	20	4
ZMIZ1 Band A	Isoform 1 of Zinc finger MIZ domain-containing protein 1	16	NOT IN CONTROL IP
ZMIZ1 Band A	ATP-dependent RNA helicase A	15	15
ZMIZ1 Band A	Isoform 2 of Heterogeneous nuclear ribonucleoprotein M	15	NOT IN CONTROL IP
ZMIZ1 Band A	cDNA FLJ59357, highly similar to Probable ATP-dependent RNA helicase DDX5	12	1.714285714
ZMIZ1 Band A	LMNB1 protein	11	1.833333333
ZMIZ1 Band A	HSPA5 protein	10	10
ZMIZ1 Band A	Isoform 1 of RNA-binding protein 14	10	1.25
ZMIZ1 Band A	cDNA FLJ54020, highly similar to Heterogeneous nuclear ribonucleoprotein U	9	1.125
ZMIZ1 Band A	insulin-like growth factor 2 mRNA-binding protein 1 isoform 2	9	NOT IN CONTROL IP
ZMIZ1 Band A	Putative uncharacterized protein LMNA	9	4.5
ZMIZ1 Band A	cDNA FLJ54408, highly similar to Heat shock 70 kDa protein 1	7	NOT IN CONTROL IP
ZMIZ1 Band A	Poly(A) binding protein, cytoplasmic 4	5	NOT IN CONTROL IP
ZMIZ1 Band A	Seipin	5	NOT IN CONTROL IP
ZMIZ1 Band A	Hornerin	4	NOT IN CONTROL IP
ZMIZ1 Band A	Isoform 5 of Double-stranded RNA-specific adenosine deaminase	3	NOT IN CONTROL IP
ZMIZ1 Band A	Histone H2A type 1-H	2	NOT IN CONTROL IP
ZMIZ1 Band A	Isoform 3 of Double-stranded RNA-specific adenosine deaminase	2	NOT IN CONTROL IP
ZMIZ1 Band A	similar to U5 snRNP-specific 200kD protein, partial	2	NOT IN CONTROL IP
ZMIZ1 Band A	heterogeneous nuclear ribonucleoprotein R isoform 4	1	NOT IN CONTROL IP
ZMIZ1 Band A	Isoform 4 of Heterogeneous nuclear ribonucleoproteins C1/C2	1	NOT IN CONTROL IP