ENCODE DCC Antibody Validation Document

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Antibody Name: DRIL1 Antibody (NB100-279)

Target: ARID3A EntrezGeneID1820

Company/ Source: Novus Biologicals

Catalog Number, database ID, laboratory: NB100-279

Lot Number: A1

Antibody Description: A synthetic peptide, which represented a portion of human Dead Ringer-Like 1 encoded within exon 8 (LocusLink ID 1820).

Target Description: This gene encodes a member of the ARID (AT-rich interaction domain) family of DNA binding proteins. It was found by homology to the Drosophila dead ringer gene, which is important for normal embryogenesis. Other ARID family members have roles in embryonic patterning, cell lineage gene regulation, cell cycle control, transcriptional regulation, and possibly in chromatin structure modification. (provided by RefSeq)

Species Target: Human

Species Host: Rabbit

Validation Method #1: Immunoprecipitation

Validation Method #2: ChIP

Purification Method: Affinity

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://www.novusbio.com/DRIL1-Antibody_

Reference (PI/ Publication Information):

Please complete the following for antibodies to histone modifications:

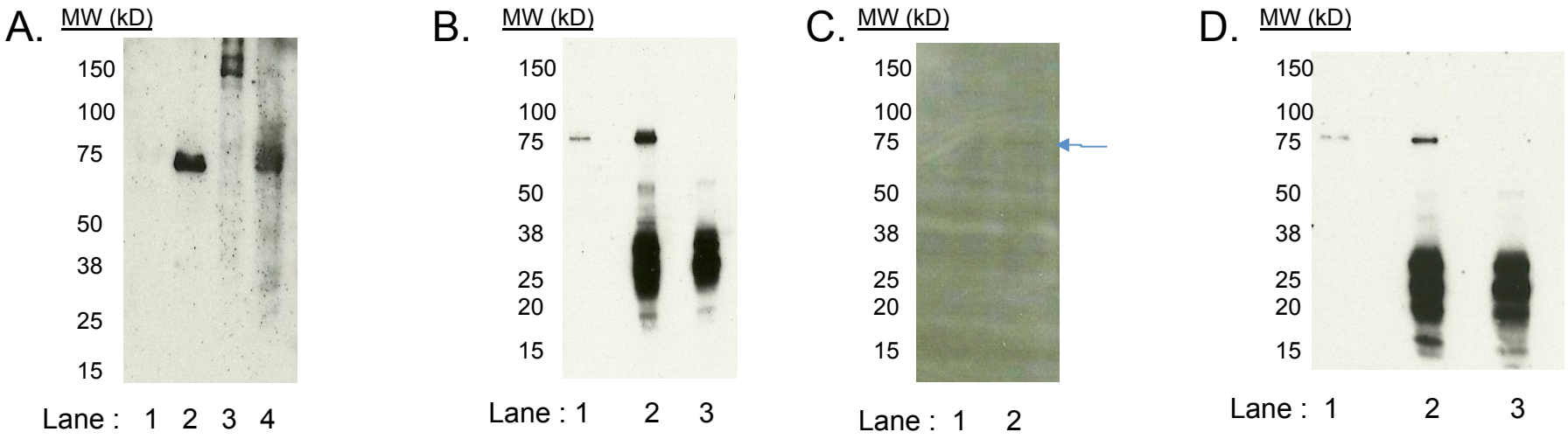
if your specifications are not listed in the drop-down box, please write-in the appropriate information

Histone Name | AA modified | AA Position | Modification

Validation #1 Analysis	COMMENT: Using antibody NB100-279, we observe a main band in Western blots on lysates from cell lines K562 and HepG2 at a position consistent with a molecular weight of ~75kD (Panel A). This band is efficiently and specifically immunoprecipitated from K562 nuclear extracts (Panel B). This mobility is somewhat slower than expected given the predicted size of ARID3A (~63kD) but is consistent with the electrophoretic mobility reported for ARID3A (see product literature for both antibodies and note the consistent mobility observed with each antibody. A band of ~75kD is also observed in Western blots on lysates from cell lines K562 using antibody sc8821 (Panel C). Western blotting with this antibody was made difficult by high background signal consistently observed on these blots (a problem routinely encountered with antibodies raised in goats from this manufacturer). To verify that this band was ARID3A, we used sc8821 to perform an immunoprecipitation and then used NB100-279 in a Western blot to analyze immunoprecipitated material (Panel D). We observed a single band immunoprecipitated from K562 cells at high efficiency that is consistent with the observed mobility of ARID3A. We conclude this antibody specifically recognizes ARID3A. Based on these data, both antibodies validated by this criterion.

Insert Validation Image (click here)

ARID3A (NB100-279) & (sc-8821)
Immunoblot / Immunoprecipitation



A. Western Blot using NB100-279 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 NB100-279. Lane1: Nuclear lysate. Lane 3: Bound material from control immunoprecipitation with rabbit IgG. . Lane 2: Bound material from immunoprecipitation with NB100-279. **C**. Western Blot using sc-8821 on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2). **D.** Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody sc-8821 and immunoblot with NB100-279. Lane1: Nuclear lysate. Lane 2: Bound material from immunoprecipitation with sc-8821. Lane 3: Bound material from control immunoprecipitation with Goat IgG. Arrow indicates band of expected size (~80kD) that is highly enriched in the specifically immunoprecipitated fraction.

Validation #2 Analysis	Comment: Because the overlap of the top 40% of peaks from datasets derived from antibodies NB100-279 and sc-8821 (compared using standard ENCODE scoring methods) exceeds the recommended threshold of 80%, both antibodies meet this criterion for validation.

Insert Validation Image (Click here)

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Validation 2: ChIPseq with alternate antibodies to the same factor

	ARID3A NB100-279	ARID3A sc-8821
Total peaks	122875	48018
% Peak overlap	**86.8**	**86.5**

Antibodies/Immunogens:
NB100-279 : Immunogen:A synthetic peptide, which represented a portion of human Dead Ringer-Like 1 encoded within exon 8
sc-8821: epitope mapping at the N-terminus of ARID3A of human origin

Comparison: K562 cells were used for ChIP-seq with antibody sc-8821 or antibody NB100-279. Peaks were called from replicate experiments using PeakSeq with a .01 q-value cut-off. Comparisons between experiments were made using these peaks according to standard ENCODE replicate comparison parameters (http://genome.ucsc.edu/ENCODE/protocols/dataStandards/ChIP_DNase_FAIRE_DNAme_v2_2011.pdf; reported is the fraction of the top 40% of peaks in one list that are found in the full list of peaks obtained with the other antibody.