

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:

Target
Description:

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*

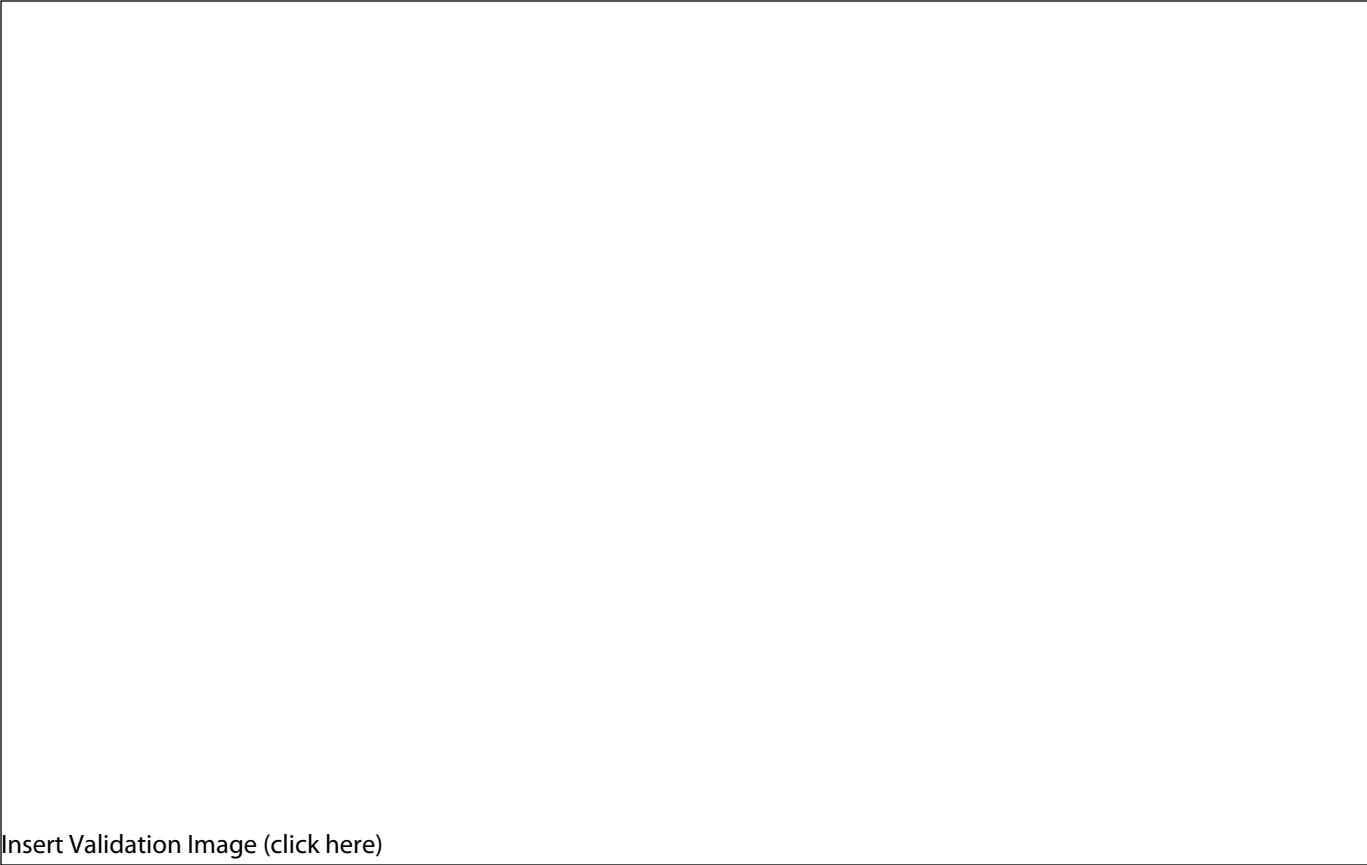
Histone Name

AA modified

AA Position

Modification

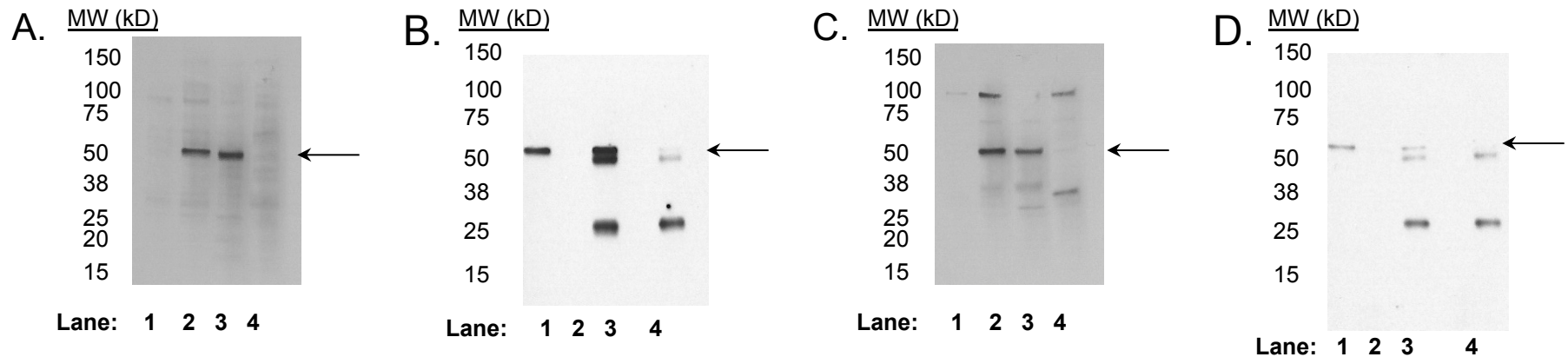
Validation #1
Analysis



Insert Validation Image (click here)

Antibody ab24550 (TBLR1) Immunoblot/ immunoprecipitation

Antibody NB600-270 (TBLR1) Immunoblot/ immunoprecipitation



A. Western blot using antibody ab24550 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 ab24550. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with ab24550. Lane 3: Bound material from immunoprecipitation with ab24550. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (56kD) that is highly enriched in the specifically immunoprecipitated fraction. **C.** Western blot using antibody NB600-270 on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4). **D.** Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody NB600-270. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with NB600-270. Lane 3: Bound material from immunoprecipitation with NB600-270. Lane 4: Bound material from control immunoprecipitation with rabbit IgG. Arrow indicates band of expected size (56kD) that is highly enriched in the specifically immunoprecipitated fraction.

Validation #2
Analysis



Insert Validation Image (Click here)

Validation 2: ChIPseq with alternate antibodies to the same factor

	TBLR1 NB600-270	TBLR1 ab24550
Total peaks	65216	38496
% Peak overlap	82.4	85.8

Antibodies/Immunogens:

NB600-270 : Peptide that maps to a region between residues 100 and 150 of human TBL1-related Protein 1

ab24550: Recombinant full length protein.

Comparison: K562 cells were used for ChIP-seq with antibody ab24550 or antibody NB600-270. 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.