

# 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

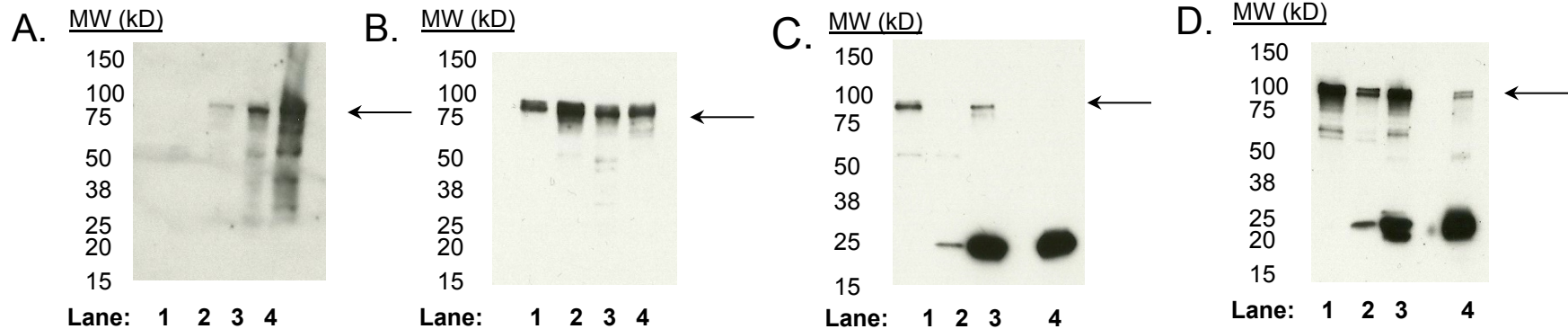
Validation #1  
Analysis



Insert Validation Image (click here)

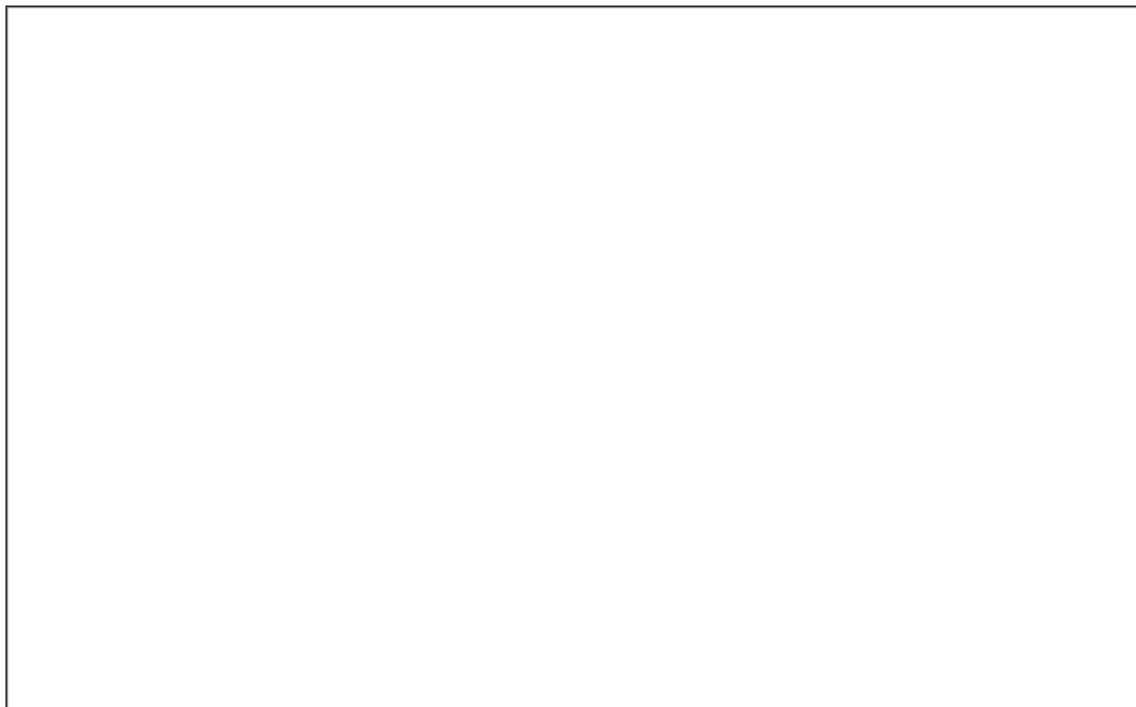


# Antibody sc13125 (UBF) and SAB1404509 (UBTF) Immunoblot/immunoprecipitation



**A.** Western blot using antibody sc13125 on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4). **B.** Western blot using antibody SAB1404509 on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4). **C.** Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody sc13125. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with sc13125. Lane 3: Bound material from immunoprecipitation with sc13125. Lane 4: Bound material from control immunoprecipitation with mouse IgG. **D.** Immunoprecipitation was performed on nuclear lysates from K562 cells using antibody SAB1404509. Lane1: Nuclear lysate. Lane 2: Unbound material from immunoprecipitation with SAB1404509. Lane 3: Bound material from immunoprecipitation with SAB1404509. Lane 4: Bound material from control immunoprecipitation with mouse IgG. Arrow indicates band of expected size (97kD) 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

	UBTF sc-13125	UBTF SAB1404509
Total peaks	34443	66880
% Peak overlap	<b>99.2</b>	<b>99.7</b>

### Antibodies/Immunogens:

**sc-13125** : raised against amino acids 1-220 of UBF of human origin

**SAB1404509**: Immunogen is UBTF (NP\_055048, 551 a.a. ~ 651 a.a) partial recombinant protein with GST tag.

**Comparison**: K562 cells were used for ChIP-seq with antibody SAB1404509 or antibody sc-13125. 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\\_DNAm\\_v2\\_2011.pdf](http://genome.ucsc.edu/ENCODE/protocols/dataStandards/ChIP_DNase_FAIRE_DNAm_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.