

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*

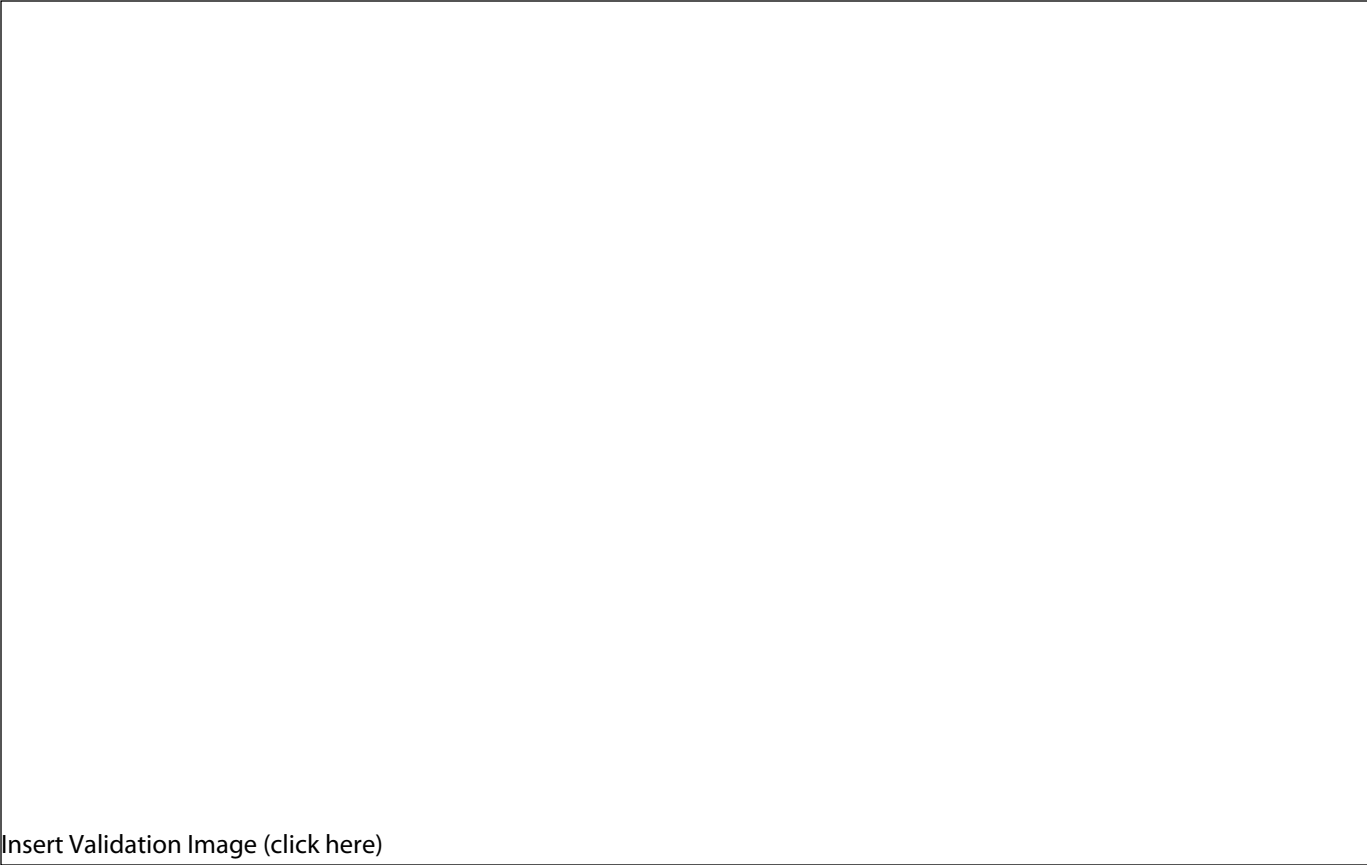
Histone Name

AA modified

AA Position

Modification

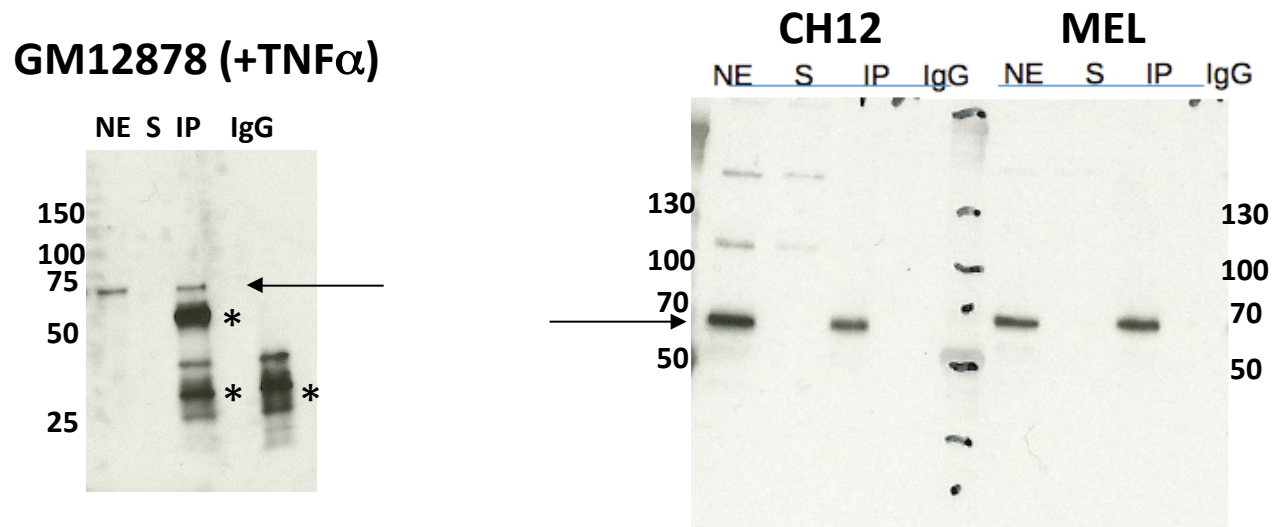
Validation #1
Analysis



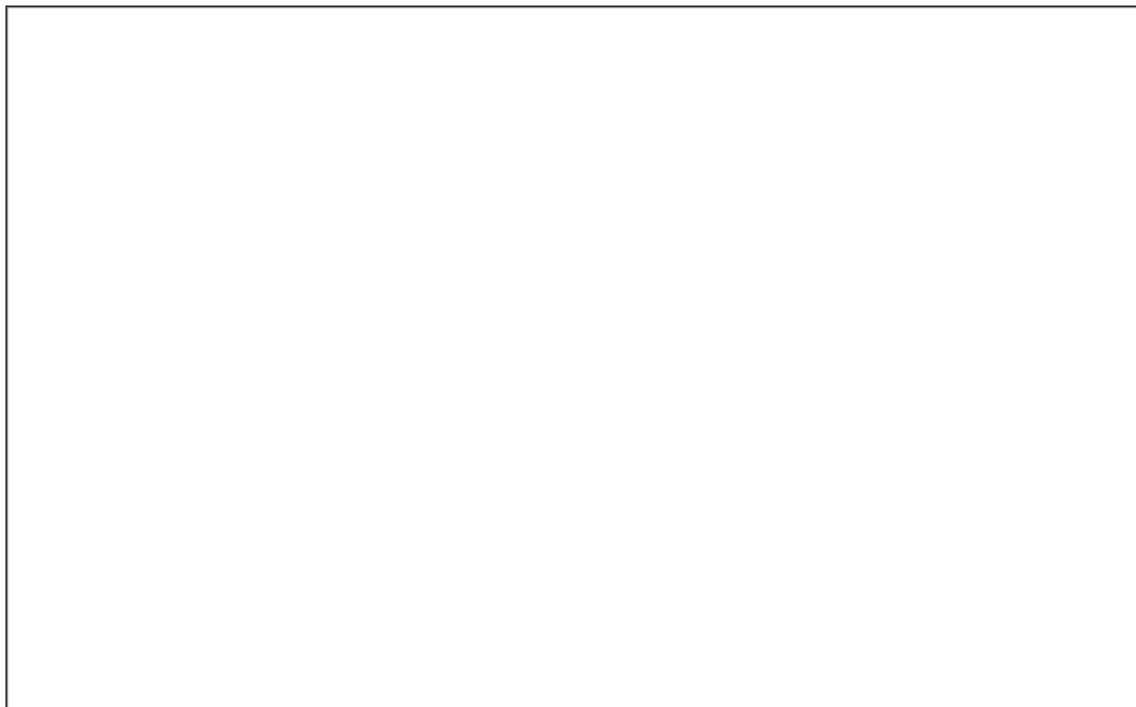
Insert Validation Image (click here)

Validation 1: Immunoblot/immunoprecipitation with sc-372 (NFKB p65)

Immunoprecipitations using sc-372 were carried out in GM12878 cells treated with TNF α (6 hrs), murine CH12 cells and murine MEL cells. NE= nuclear lysate, S= supernatant from immunoprecipitation with sc-372, IP= bound material from immunoprecipitation with sc-372, IgG= bound material from immunoprecipitation with control IgG. Arrows indicate band consistent with the expected size of NFKB p65. Bands indicated by * in GM12878 immunoprecipitation are IgG heavy and light chains. These bands are absent from CH12 and MEL immunoprecipitations because antibody was crosslinked to Protein A beads prior to use in immunoprecipitation. Molecular weights are indicated in kD.



Validation #2
Analysis



Insert Validation Image (Click here)

Table 1. NFKB motif enrichment.

Cell Line	Motif Enrichment (log2)	Enrichment p-value (-log10)	Fraction of peaks containing motif
NFKB_GM10847	3.38588 (NF-kappaB_known1)	1383.46 (NF-kappaB_known1)	0.410202 (NF-kappaB_known5)
NFKB_GM12878	3.40875 (NF-kappaB_known1)	1847.76 (NF-kappaB_known1)	0.331598 (NF-kappaB_known5)
NFKB_GM12878	3.50712 (NF-kappaB_known1)	1953.32 (NF-kappaB_known1)	0.336786 (NF-kappaB_known5)
NFKB_GM12891	2.98034 (NF-kappaB_known1)	3158.79 (NF-kappaB_known1)	0.311203 (NF-kappaB_known5)
NFKB_GM12892	3.58808 (NF-kappaB_known1)	1025.9 (NF-kappaB_known1)	0.485587 (NF-kappaB_known5)
NFKB_GM15510	2.1634 (NF-kappaB_known5)	1034.71 (NF-kappaB_known5)	0.206633 (NF-kappaB_known5)
NFKB_GM18505	3.48915 (NF-kappaB_known1)	1451.5 (NF-kappaB_known1)	0.383419 (NF-kappaB_known5)
NFKB_GM18526	3.9191 (NF-kappaB_known1)	446.627 (NF-kappaB_known1)	0.642955 (NF-kappaB_known5)
NFKB_GM18951	2.63442 (NF-kappaB_known1)	1560.49 (NF-kappaB_known1)	0.265892 (NF-kappaB_known5)
NFKB_GM19099	3.44391 (NF-kappaB_known1)	1719.2 (NF-kappaB_known1)	0.449522 (NF-kappaB_known5)
NFKB_GM19193	3.49646 (NF-kappaB_known1)	1175.42 (NF-kappaB_known1)	0.477133 (NF-kappaB_known5)

Figure 2. Motif consensus sequences and position weight matrices for highly enriched NFKB motif.

NFKB_known1



>NF-kappaB_known1 NF-kappaB-(p50)_transfac_M00051 +

Position/
Consensus

	A	C	G	T
G	0.000000	0.000000	1.000000	0.000000
G	0.000000	0.000000	1.000000	0.000000
G	0.000000	0.000000	1.000000	0.000000
G	0.111111	0.000000	0.888889	0.000000
A	0.888888	0.055556	0.000000	0.055556
K	0.000000	0.000000	0.166667	0.833333
Y	0.000000	0.388889	0.055556	0.555555
C	0.000000	0.888889	0.000000	0.111111
C	0.000000	1.000000	0.000000	0.000000
C	0.000000	0.944444	0.055556	0.000000

NFKB_known5



>NF-kappaB_known5 RELA_jaspar_MA0107.1 +

Position/
Consensus

	A	C	G	T
B	0.000000	0.222222	0.611111	0.166667
G	0.000000	0.000000	0.944444	0.055556
G	0.000000	0.000000	1.000000	0.000000
R	0.611111	0.000000	0.388889	0.000000
V	0.555555	0.166667	0.222222	0.055556
T	0.111111	0.000000	0.000000	0.888889
T	0.000000	0.000000	0.000000	1.000000
T	0.000000	0.111111	0.000000	0.888889
C	0.000000	1.000000	0.000000	0.000000
C	0.000000	1.000000	0.000000	0.000000