ENCODE DCC Antibody Validation Document

Date of Submission: 1/4/12

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Antibody Name: c-Jun (H-79) Antibody: sc-1694

Target: Jun (EntrezGene ID 3725)

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-1694

Lot Number: C2206

Antibody Description: rabbit polyclonal IgG, 200 µg/ml
epitope corresponding to amino acids 1-79 mapping at the N-terminus of c-Jun p39 of human origin
recommended for detection of c-Jun p39 of mouse, rat, human and Xenopus origin

Target Description: This gene is the putative transforming gene of avian sarcoma virus 17. It encodes a protein which is highly similar to the viral protein, and which interacts directly with specific target DNA sequences to regulate gene expression. This gene is intronless and is mapped to 1p32-p31, a chromosomal region involved in both translocations and deletions in human malignancies.

Species Target: Human

Species Host: Rabbit

Validation Method #1: Immunoprecipitation

Validation Method #2: Motif Enrichment

Purification Method: Other Purification Method

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://www.scbt.com/datasheet-1694-c-jun-

Reference (PI/ Publication Information):

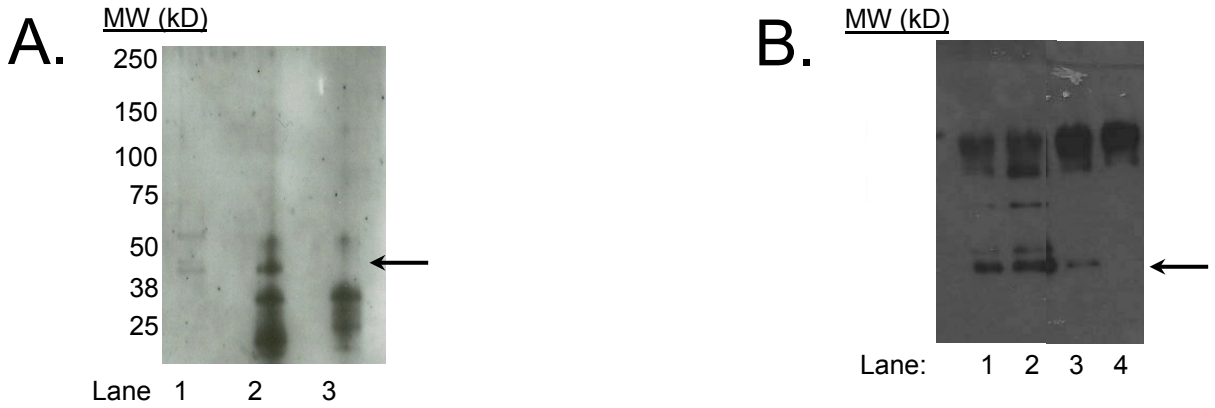
Please complete the following for antibodies to histone modifications:
if your specifications are not listed in the drop-down box,
please write-in the appropriate information

Histone Name | AA modified | AA Position | Modification

Validation #1 Analysis	Comment: A major band with a mobility consistent with the size expected for c-Jun (~36kD; indicated by arrows) is specifically immunoprecipitated from nuclear lysates from K562 and HeLa S3 cells. Therefore, sc-1694 meets this criterion for validation.

Insert Validation Image (click here)

sc-1694 (c-Jun) Immunoprecipitation



A. Immunoprecipitation of c-Jun from K562 cells using sc-1694. Lane 1: input nuclear lysate, Lane 2: material immunoprecipitated with sc-1694, Lane 3: material immunoprecipitated using control IgG. **B.** Immunoprecipitation of c-Jun from HeLa S3 cells using sc-1694. Lanes 1-2; material immunoprecipitated with sc-1694, lanes 3-4: material immunoprecipitated using control IgG. 1ug of antibody was used for immunoprecipitations in lanes 1 and 3, 2ug in lanes 2 and 4.

Validation #2 Analysis	Calculations were done by Pouya Kheradpour using a collection of known motifs available at http://www.broadinstitute.org/%7Epouyak/motif-disc/human/. Table 1 shows the fold-enrichments, enrichment p-values and fraction of peaks which contain the motif. The motif which produced the largest value for each criterion is shown in Table 1. Note that while the maximally enriched motifs may differ from the motif with the highest enrichment p-values and the most represented motif, the motifs are highly similar (Figure 1) and thus all values are similar between motifs. Motifs were identified using a matching stringency corresponding to 4-6 (6-mer). Peaks identified by IDR (1% cutoff) were used in the analysis and +/-50bp from peak centers were considered. Enrichments are for a given motif vs. a background consisting of +/- 50bp from the centers of all DnaseI hypersensitive peaks. Repeat mask/simple repeats from UCSC and all gencode v7 exons (including non-protein coding genes) were excluded from the analysis. Comparison to shuffle motifs were used to correct for compositional bias. Enrichment is the corrected # of motifs in ChIP peaks/corrected # of motifs in DNaseI peaks. The current ENCODE standard calls for >4-fold enrichment and >10% motif representation for this criteria to be used for validation. The 7 c-Jun datasets presented here exceed these thresholds and sc-1694 is considered validated.

Insert Validation Image (Click here)

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- **Table 1.** c-Jun motif enrichment.

Cell Line	Motif Enrichment (log2)	Enrichment p-value (-log10)	Fraction of peaks containing motif
H1-hESC	2.1343 (AP-1_known2)	61.5585 (AP-1_known3)	0.36747 (AP-1_known2)
HeLa S3	2.49159 (AP-1_known2)	1447.53 (AP-1_known1)	0.589381 (AP-1_known2)
HUVEC	2.47206 (AP-1_known2)	1726.43 (AP-1_known1)	0.546169 (AP-1_known2)
K562	2.63146 (AP-1_known2)	684.812 (AP-1_known1)	0.586961 (AP-1_known2)
K562 (IFNα- 6hrs)	2.50294 (AP-1_known2)	388.382 (AP-1_known1)	0.565164 (AP-1_known2)
K562 (IFNγ- 30 min)	2.3547 (AP-1_known2)	532.138 (AP-1_known1)	0.541051 (AP-1_known2)
K562 (IFNγ- 6hrs)	2.37234 (AP-1_known2)	406.571 (AP-1_known1)	0.585404 (AP-1_known2)

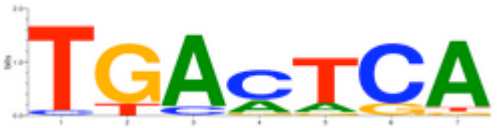
- **Figure 2.** Motif consensus sequences and position weight matrices for highly enriched c-Jun motif.

AP1_known1

>AP-1_known1 AP-1_transfac_M00173 +

Position/ Consensus	A	C	G	T
R	0.428571	0.071429	0.500000	0.000000
V	0.214286	0.357143	0.428571	0.000000
T	0.000000	0.000000	0.000000	1.000000
G	0.000000	0.000000	1.000000	0.000000
A	1.000000	0.000000	0.000000	0.000000
C	0.000000	0.928571	0.000000	0.071429
T	0.142857	0.071429	0.142857	0.642857
M	0.285714	0.428571	0.142857	0.142857
M	0.571428	0.285714	0.071429	0.071429
K	0.142857	0.142857	0.500000	0.214286
W	0.428571	0.071429	0.071429	0.428571

AP1_known2



>AP-1_known2 AP1_jaspar_MA0099.2 +

	A	C	G	T
T	0.000000	0.055556	0.000000	0.944444
K	0.000000	0.000000	0.833333	0.166667
A	0.888889	0.111111	0.000000	0.000000
M	0.277778	0.666666	0.055556	0.000000
W	0.166667	0.000000	0.055556	0.777777
S	0.000000	0.833333	0.166667	0.000000
A	0.888888	0.000000	0.055556	0.055556

AP1_known3

>AP-1_known3 AP-1_transfac_M00926 -

	A	C	G	T
V	0.403361	0.252101	0.243697	0.100840
T	0.000000	0.000000	0.008403	0.991597
G	0.016807	0.008403	0.941177	0.033613
A	0.991597	0.000000	0.008403	0.000000
C	0.084034	0.722689	0.142857	0.050420
T	0.142857	0.109244	0.109244	0.638655
H	0.184874	0.546218	0.042017	0.226891
A	0.714285	0.159664	0.058824	0.067227