ENCODE DCC Antibody Validation Document

Date of Submission: 10/28/11

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Antibody Name: C/EBP β (C-19) Antibody: sc-150

Target: CEBPbeta (EntrezGeneID 1051)

Company/ Source: Santa Cruz BIotechnology

Catalog Number, database ID, laboratory: sc-150

Lot Number: J0110

Antibody Description:
rabbit polyclonal IgG, 200 µg/ml;
epitope mapping at the C-terminus of C/EBP β of rat origin
recommended for detection of C/EBP β of mouse, rat and human origin

Target Description:
The protein encoded by this intronless gene is a bZIP transcription factor which can bind as a homodimer to certain DNA regulatory regions. It can also form heterodimers with the related proteins CEBP-alpha, CEBP-delta, and CEBP-gamma. The encoded protein is important in the regulation of genes involved in immune and inflammatory responses and has been shown to bind to the IL-1 response element in the IL-6 gene, as well as to regulatory regions of several acute-phase and cytokine genes. In addition, the encoded

Species Target: Human

Species Host: Rabbit

Validation Method #1: Immunoprecipitat

Validation Method #2: Motif Enrichment

Purification Method: Affinity

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://www.scbt.com/datasheet-150-c-ebp-b

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:

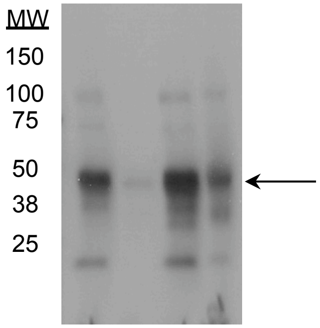
Comment: In Western blotting experiments, we observe one or two bands of ~40kD in all cell lines tested and these bands constitute the majority of signal observed. In panel B, we demonstrate that the smaller band is efficiently immunoprecipitated, while the larger band is immunoprecipitated at much lower levels. We note that multiple bands of sizes larger than the expected 36kD band for CEBP have been observed with this antibody (see product literature and, for example, Shen, et.al. J Biol Chem. 2006 281(34):24138-48) and other antibodies (for example, Guerzoni, et.al. Blood. 2006 107: 4080-4089). Therefore, this antibody meets ENCODE standards for validation by this criterion.

Validation #1
Analysis

Insert Validation Image (click here)

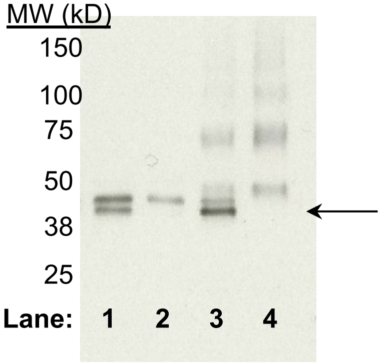
Validation 1: Immunoblot/immunoprecipitation with sc-150 (CEBP β)

A.



Arrow indicates band consistent with expected size (~40 kD) of CEBP β in nuclear lysates from (left to right): K562, GM12878, HeLaS3, and HepG2 cell lines.

B.



Arrow indicates immunoprecipitated band of expected size from HeLa S3 nuclear lysates. Lane 1= HeLa S3 nuclear lysate, Lane 2= supernatant from HeLa S3 immunoprecipitation (IP), Lane 3= bound material from HeLa S3 IP, Lane 4= bound material from control IgG IP from HeLa S3.

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 4 CEBP/b datasets presented here exceed these thresholds and the antibody is considered validated

Insert Validation Image (Click here)

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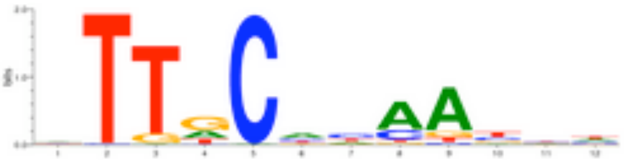
Table 1. CEBP/b motif enrichment.

Cell Line	Motif Enrichment (log2)	Enrichment p-value (-log10)	Fraction of peaks containing motif
HeLa-S3	2.86562 (CEBPB_known5)	5053.02 (CEBPB_known5)	0.37903 (CEBPB_known1)
HepG2	3.3304 (CEBPB_known5)	2358.53 (CEBPB_known5)	0.433258 (CEBPB_known5)
HepG2 (forskolin)	3.36494 (CEBPB_known5)	5209.69 (CEBPB_known5)	0.47111 (CEBPB_known5)
K562	3.24554 (CEBPB_known5)	3812.18 (CEBPB_known5)	0.429311 (CEBPB_known5)

Figure 2. Motif consensus sequences and position weight matrices for highly enriched CEBP/b motifs.

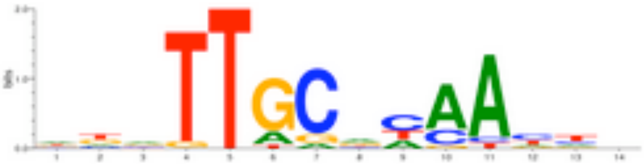
CEBP/b_known1



`>CEBPB_known1  C/EBP_transfac_M00912 +`

Position/ Consensus	A	C	G	T
N	0.359551	0.213483	0.202247	0.224719
T	0.000000	0.011236	0.000000	0.988764
T	0.011236	0.000000	0.101124	0.887640
D	0.269663	0.022472	0.505618	0.202247
C	0.011236	0.988764	0.000000	0.000000
H	0.426966	0.258427	0.112360	0.202247
H	0.224719	0.393258	0.089888	0.292135
M	0.651685	0.235955	0.056180	0.056180
A	0.764044	0.033708	0.112360	0.089888
B	0.089888	0.269663	0.202247	0.438202
H	0.213483	0.280899	0.157303	0.348315
H	0.303371	0.280899	0.089888	0.325843

CEBP/b_known5



`>CEBPB_known5  C/EBPbeta_transfac_M00117 +`

Position/ Consensus	A	C	G	T
D	0.352941	0.117647	0.235294	0.294118
K	0.117647	0.117647	0.352941	0.411765
V	0.352941	0.235294	0.294118	0.117647
T	0.000000	0.000000	0.058824	0.941176
T	0.000000	0.000000	0.000000	1.000000
R	0.176471	0.000000	0.764705	0.058824
C	0.058824	0.823529	0.117647	0.000000
D	0.352941	0.117647	0.352941	0.176471
H	0.235294	0.470588	0.000000	0.294118
M	0.705882	0.235294	0.058824	0.000000
A	0.882352	0.058824	0.000000	0.058824
Y	0.117647	0.411765	0.117647	0.352941
H	0.176471	0.235294	0.117647	0.470588
N	0.235294	0.294118	0.294118	0.176471