ENCODE DCC Antibody Validation Document

Date of Submission: 1/4/12

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Antibody Name: Stat1α p91 (C-24) Antibody: sc-345

Target: STAT1 (EntrezGene ID 6772)

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-345

Lot Number: D2908

Antibody Description:
rabbit polyclonal IgG, 200 µg/ml
epitope mapping at the C-terminus of Stat1α p91 of human origin
recommended for detection of Stat1α p91 of mouse, rat and human origin

Target Description:
Signal transducer and activator of transcription that mediates signaling by interferons (IFNs). Following type I IFN (IFN-alpha and IFN-beta) binding to cell surface receptors, Jak kinases (TYK2 and JAK1) are activated, leading to tyrosine phosphorylation of STAT1 and STAT2. The phosphorylated STATs dimerize, associate with ISGF3G/IRF-9 to form a complex termed ISGF3 transcription factor, that enters the nucleus.

Species Target: Human

Species Host: Rabbit

Validation Method #1: Western Blot

Validation Method #2: Motif Enrichment

Purification Method: Affinity

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: lhttp://www.scbt.com/datasheet-345-stat1al

Reference (PI/ Publication Information):

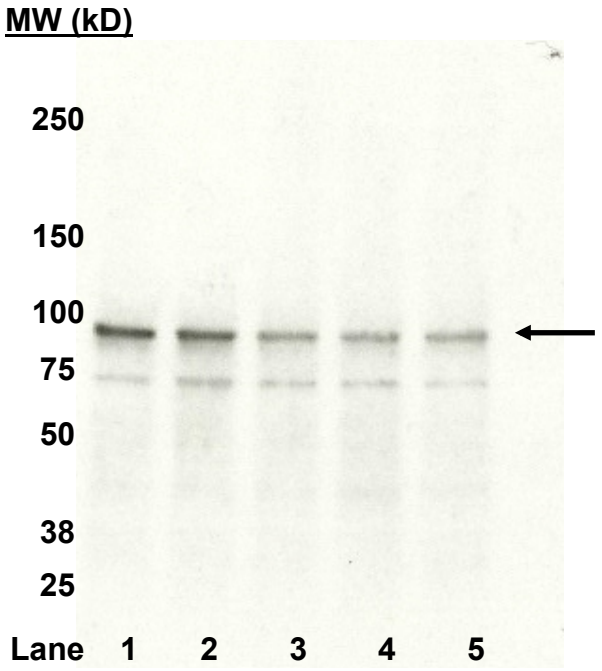
Please complete the following for antibodies to histone modifications:
if your specifications are not listed in the drop-down box,
please write-in the appropriate information

Histone Name | AA modified | AA Position | Modification

Validation #1 Analysis	Comment: A predominant band consistent with the size expected for STAT1 (~91kD, indicated by arrow) is observed in HeLa S3 lysates using sc-345. Therefore, sc-345 meets this criterion for validation.

Insert Validation Image (click here)

sc-345 (STAT1) Immunoblot



Western blot using sc-345. Lanes contain nuclear lysates from HeLa S3 cells treated with interferon-γ for 0 minutes (lane 1), 10 min. (lane 2), 20 min. (lane 3), 30 min. (lane 4), 60 min. (lane 5), Arrow indicates band consistent with the size expected for STAT1.

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 5 STAT1 and STAT2 datasets presented here exceed these thresholds and the antibody is considered validated. In addition, we note that this antibody is also validated based on the overlap between data obtained with antibody sc-345 (STAT1) and antibody sc-476 (STAT2), which are known to form a complex upon interferon stimulation. Details can be found in the validation document for sc-476.

Insert Validation Image (Click here)

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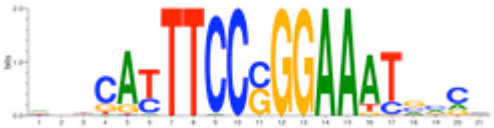
- **Table 1.** STAT1/2 motif enrichment.

Cell Line	Motif Enrichment (log2)	Enrichment p-value (-log10)	Fraction of peaks containing motif
STAT1_HeLa-S3_IFNγ30	3.82013 (STAT_known10)	1652.68 (STAT_known12)	0.339567 (STAT_known12)
STAT1_K562_IFNα30	4.06578 (STAT_known10)	171.64 (STAT_known6)	0.322124 (STAT_known12)
STAT1_K562_IFNγ30	5.05755 (STAT_known10)	605.77 (STAT_known6)	0.588235 (STAT_known12)
STAT1_K562_IFNγ6h	4.74521 (STAT_known10)	535.18 (STAT_known6)	0.46729 (STAT_known12)
STAT2_K562_IFNα30	3.19162 (STAT_known10)	172.42 (STAT_known12)	0.198288 (STAT_known12)

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

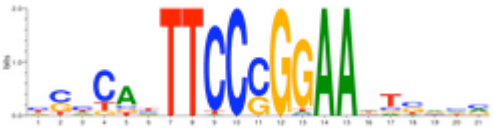
STAT_known6



>STAT_known6 STAT3_transfac_M00225 -

Position/ Consensus	A	C	G	T
D	0.401000	0.160000	0.243000	0.196000
N	0.321000	0.224000	0.182000	0.273000
N	0.186813	0.279720	0.186813	0.346653
S	0.030000	0.700000	0.193000	0.077000
A	0.826000	0.018000	0.138000	0.018000
Y	0.040000	0.327000	0.000000	0.633000
T	0.000000	0.000000	0.000000	1.000000
T	0.000000	0.000000	0.000000	1.000000
C	0.018000	0.982000	0.000000	0.000000
C	0.000000	1.000000	0.000000	0.000000
S	0.000000	0.500000	0.500000	0.000000
G	0.000000	0.000000	1.000000	0.000000
G	0.000000	0.000000	1.000000	0.000000
A	1.000000	0.000000	0.000000	0.000000
A	1.000000	0.000000	0.000000	0.000000
A	0.806000	0.000000	0.068000	0.126000
Y	0.000000	0.213000	0.008000	0.779000
V	0.219000	0.279000	0.452000	0.050000
V	0.333000	0.394000	0.175000	0.098000
V	0.203407	0.592184	0.180361	0.024048
V	0.328328	0.307307	0.203203	0.161161

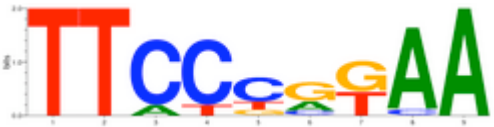
STAT_known10



>STAT_known10 STAT1_transfac_M00224 -

A	C	G	T
Y 0.159840	0.396603	0.125874	0.317682
S 0.015015	0.492492	0.367367	0.125125
Y 0.159000	0.466000	0.127000	0.248000
Y 0.000000	0.696697	0.105105	0.198198
A 0.667000	0.111000	0.111000	0.111000
H 0.254745	0.294705	0.087912	0.362637
T 0.000000	0.000000	0.000000	1.000000
T 0.000000	0.000000	0.000000	1.000000
C 0.012012	0.949950	0.000000	0.038038
C 0.000000	1.000000	0.000000	0.000000
S 0.000000	0.675000	0.325000	0.000000
G 0.000000	0.000000	1.000000	0.000000
G 0.032000	0.020000	0.909000	0.039000
A 1.000000	0.000000	0.000000	0.000000
A 1.000000	0.000000	0.000000	0.000000
D 0.331668	0.093906	0.262737	0.311688
T 0.118881	0.150849	0.134865	0.595405
B 0.064064	0.381381	0.379379	0.175175
V 0.351297	0.197605	0.312375	0.138723
H 0.298298	0.372372	0.097097	0.232232
V 0.321000	0.419000	0.180000	0.080000

STAT_known12



>STAT_known12 STATx_transfac_M00223 +

A	C	G	T
T 0.000000	0.000000	0.000000	1.000000
T 0.000000	0.000000	0.000000	1.000000
C 0.142857	0.857143	0.000000	0.000000
C 0.000000	0.857143	0.000000	0.142857
Y 0.000000	0.642857	0.142857	0.214286
R 0.285714	0.142857	0.571429	0.000000
K 0.000000	0.000000	0.571429	0.428571
A 0.928571	0.071429	0.000000	0.000000
A 1.000000	0.000000	0.000000	0.000000