ENCODE DCC Antibody Validation Document

Date of Submission: 1/4/12

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Antibody Name: Stat2 (C-20) Antibody: sc-476

Target: STAT2 (EntrezGene ID 6773)

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-476

Lot Number: B1908

Antibody Description: rabbit polyclonal IgG, 200 µg/ml
epitope mapping at the C-terminus of Stat2 of human origin
recommended for detection of Stat2 p113 of human origin

Target Description: Signal transducer and activator of transcription that mediates signaling by type I IFNs (IFN-alpha and IFN-beta). Following type I IFN 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. ISGF3 binds

Species Target: Human

Species Host: Rabbit

Validation Method #1: Immunoprecipitation

Validation Method #2: ChIP

Purification Method: Affinity

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: lhttp://www.scbt.com/datasheet-476-stat2-c-

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

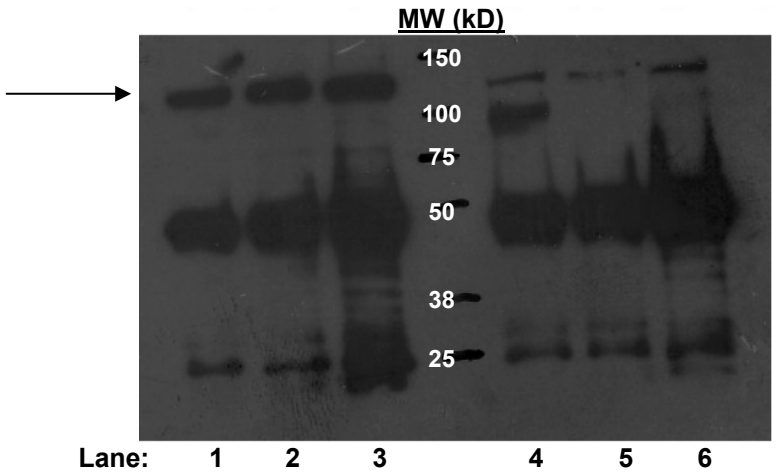
Histone Name | AA modified | AA Position | Modification

Validation #1 Analysis

Comment: A single band consistent with the size expected for STAT2 (~113kD, indicated by arrow) is observed in material immunoprecipitated from HeLa S3 lysates using sc-476. This band is efficiently and specifically immunoprecipitated. Therefore, sc-476 meets this criterion for validation.

Insert Validation Image (click here)

sc-476 (STAT2)
Immunoprecipitation



Immunoprecipitation of STAT2 from HeLa S3 cells using sc-476. Lanes 1-3; material immunoprecipitated with sc-476, lanes 4-6: material immunoprecipitated using control IgG. 1ug of antibody was used for immunoprecipitations in lanes 1 and 4, 2ug in lanes 2 and 5, 4 ug in lanes 3 and 6. Arrow indicates band consistent with the expected size of STAT2.

Validation #2 Analysis

Comment: STAT1 and STAT2 are known to homodimerize upon activation by interferon- (see for example Li et. al., J. Biol. Chem. 271(10);5790-4). Due to the high level of overlap between peaks called from data obtained using antibody sc-345 (STAT1) and antibody sc-476 (STAT2), both antibodies meet this criterion for validation.

We also note that this antibody meets the ENCODE motif enrichment standard for validation based on the 9.2-fold enrichment (p-value < 10-172) of a STAT motif in the dataset generated in interferon-stimulated K562 cells. Details can be found in the validation document for sc-345.

Insert Validation Image (Click here)

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Validation 2: Comparison to alternate member of known biochemical complex

Interferon α treatment	STAT1 Peak count	STAT2 Peak count	Fraction overlapping
30 min.	258	427	**0.99**
6 hr.	344	610	**1**

K562 cells treated with Interferon-α for the indicated times were used for ChIP-seq with antibody sc-345 (STAT1) or antibody sc-476 (STAT2). Peaks were called from replicate experiments using PeakSeq with a .01 q-value. Comparisons between experiments were made according to standard ENCODE replicate comparison parameters (http://genome.ucsc.edu/ENCODE/protocols/dataStandards/ChIP_DNase_FAIRE_DNAme_v2_2011.pdf; reported above is the fraction of the top 40% of STAT1 peaks that are found in the full list of STAT2 peaks. The reciprocal comparison was made with similar results.).