ENCODE DCC Antibody Validation Document

Date of Submission: 08/21/2012

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Antibody Name: JunD

Target: antibody raised against a peptide mapping at the C-

Company/ Source: Santa Cruz Biotechnology

Catalog Number, database ID, laboratory: sc-74

Lot Number: E2808

Antibody Description: Rabbit polyclonal antibody raised against a peptide mapping at the C-terminus of Jun D of mouse origin

Target Description: The protein encoded by this intronless gene is a member of the JUN family, and a functional component of the AP1 transcription factor complex. It has been proposed to protect cells from p53-dependent senescence and apoptosis. Alternate translation initiation site usage results in the production of different isoforms. (RefSeq). Entrez geneID: 3727, 35 kDa

Species Target: Human

Species Host: Rabbit

Validation Method #1: Western Blot

Validation Method #2: IP-Mass Spec

Purification Method:

Polyclonal/ Monoclonal: Polyclonal

Vendor URL: http://datasheets.scbt.com/sc-74.pdf

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

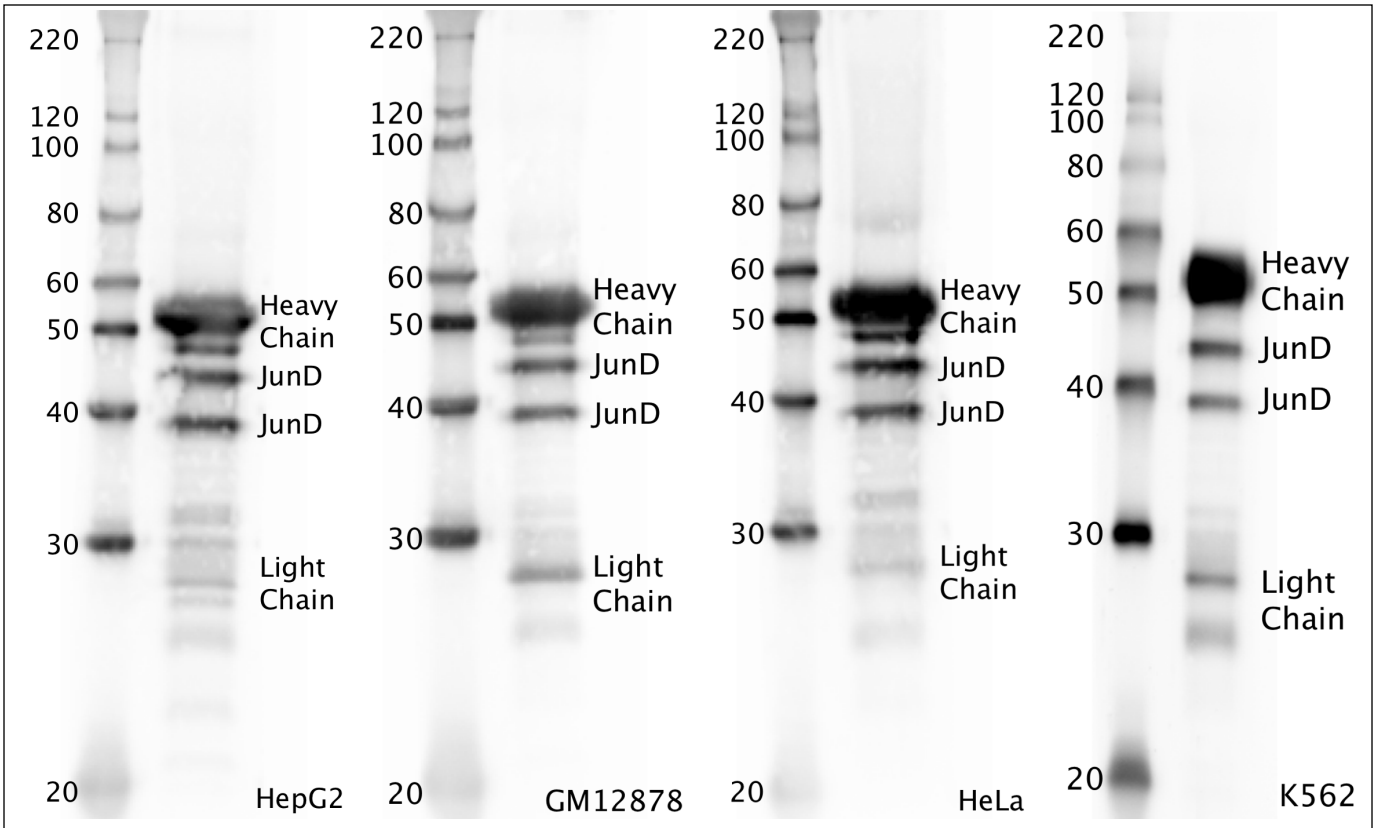
Histone Name: AA modified: AA Position: Modification:

Validation #1
Analysis

Western blot protocol:
Whole cell lysate was immunoprecipitated using primary antibody, and the IP fraction was loaded on a 12% acrylamide gel and separated with a Bio-Rad PROTEAN II xi system. After separation, the samples were transferred to a nitrocellulose membrane with an Invitrogen iBlot system. Blotting with primary (same as that used for IP) and secondary HRP-conjugated antibodies was performed on an Invitrogen BenchPro 4100 system. Visualization was achieved using SuperSignal West Femto solution (Thermo Scientific).

Results: Bands of expected size visualized, representing strongest signals in the lane.

Figure legend: IP-western with sc-74 in whole cell lysates of HepG2, GM12878, HeLa, and K562. JunD bands are indicated, as are heavy and light chains of IgG.



Insert Validation Image (click here)

Validation #2 Analysis	IP followed by mass spectrometry: Briefly, HepG2 whole cell lysates were immunoprecipitated using primary antibody, and the IP fraction was loaded on a 12% acrylamide gel and separated with a Bio-Rad PROTEAN II xi system. Gel was stained with Coomassie Blue in order to visualize marker bands. Gel fragments corresponding to the bands indicated above in the western blot image were excised and sent to the University of Alabama at Birmingham Cancer Center Mass Spectrometry/Proteomics Shared Facility. There the samples were run on an LTQ XL Linear Ion Trap Mass Spectrometer by LC-ESI-MS/MS. Peptides were identified using MASCOT and Scaffold tandem mass spectra analysis, with probability based matching at FDR < 0.05. As per ENCODE data standards, all Scaffold results are attached (ENCODE_HAIB_JunD_sc74_MassSpec.pdf), including common contaminants. Target protein is highlighted in each of the two samples corresponding to the two JunD bands.

Insert Validation Image (Click here)

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