ENCODE Antibody Validation Documentation
Transcription factor: basic helix-loop-helix family, member e40 (GeneID 8553)

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Transcription factor: BHLHE40 (GeneID 8553; ~45 kDa)

Antibody: DEC1 (S-8), Santa Cruz Biotechnology (sc-101023)
Mouse monoclonal, raised against recombinant DEC1 of human origin
Web: http://www.scbt.com/datasheet-101023-dec1-s-8-antibody.html

Validation 1: Immunoblot Analysis

For an antibody to meet ENCODE validation standards, a single band of the predicted size, or a band of no less than half the total signal, must be detected in a lane on a Western blot.

a. Vendor immunoblot analysis

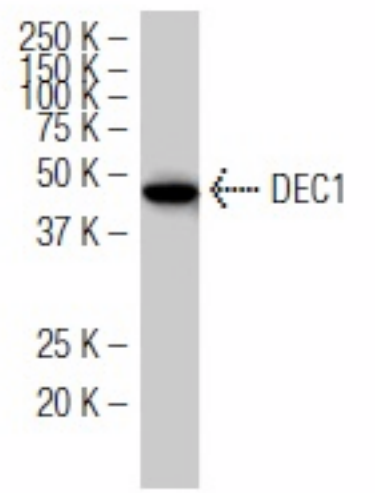

Figure Legend: Western blot analysis of DEC1 expression in HepG2 whole cell lysate.

b. Myers Lab immunoblot analysis

Western blot protocol

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. After separation, the samples were transferred to a nitrocellulose membrane using a Bio-Rad Trans-Blot Electrophoretic Transfer system. Standard western blot protocol was used to probe the membrane with the primary antibody (same antibody as used for IP), and an HRP-conjugated secondary antibody and SuperSignal West Femto solution (Thermo Scientific) were used to detect the immunoprecipitated proteins.

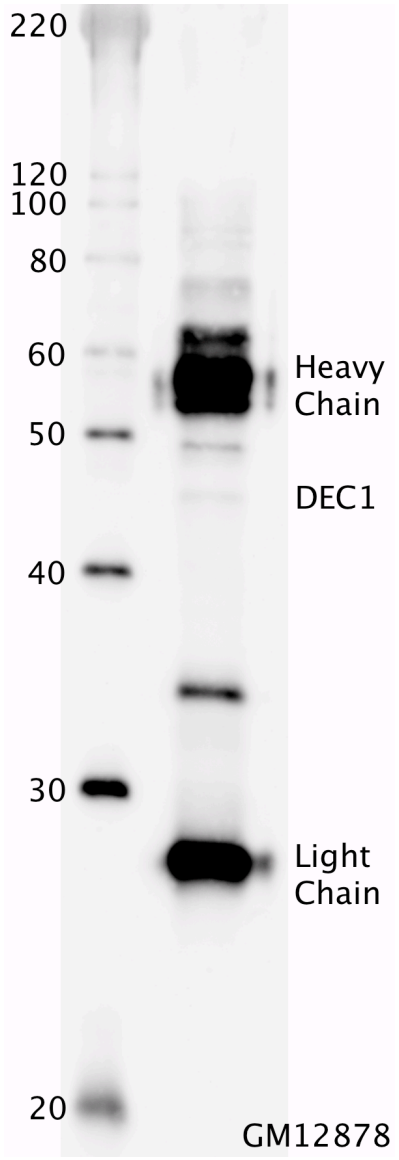

Figure Legend: BHLHE40 immunoblot: IP-western with sc-101023 DEC1 antibody in whole cell lysate of GM12878. Heavy

chain and light chain of IgG are indicated, and DEC1 band is indicated at ~45 kDa.

Validation 2: In progress