ENCODE Antibody Validation Documentation
Transcription factor: early growth response 1 (GeneID 1958)

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Transcription factor: EGR1 (GeneID 1958; ~57 kDa)

Antibody: EGR1 (588), Santa Cruz Biotechnology (sc-110)
Rabbit polyclonal, epitope mapping at the C-terminus of EGR1 of human origin
Web: http://www.scbt.com/datasheet-110-egr-1-588-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

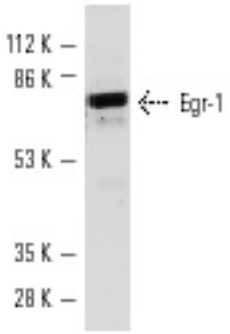

Figure Legend: Western blot analysis of EGR1 expression in NIH/3T3 whole cell lysate.

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.

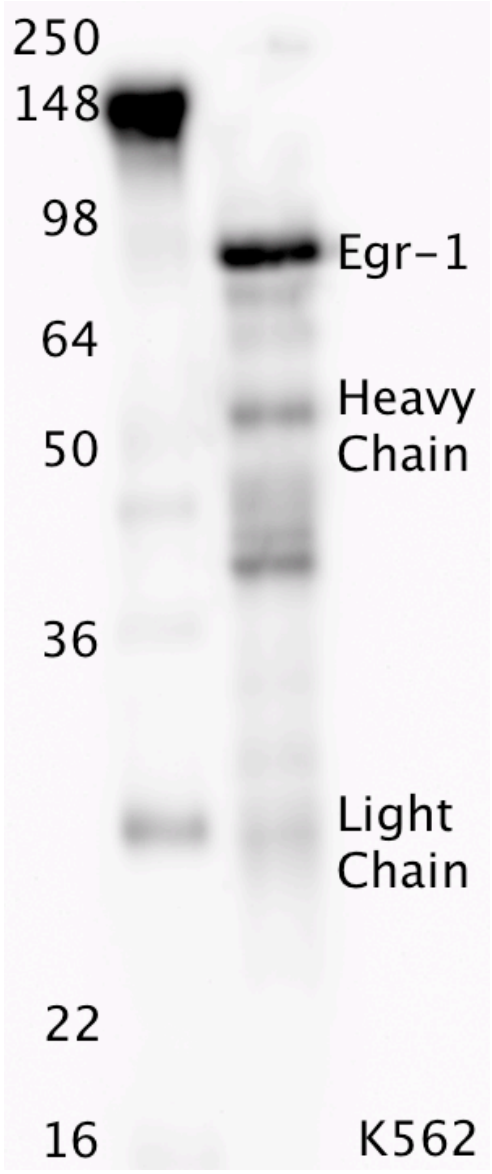

Figure Legend: EGR1 immunoblot: IP-western with sc-110 EGR1 antibody in whole cell lysate of K562. Heavy chain and light chain of IgG are indicated, and EGR1 band is indicated at ~80 kDa.

Validation 2: In progress