ENCODE Antibody Validation Documentation
Transcription factor: transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47) (GeneID 6929)

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Transcription factor: TCF3 (GeneID 6929; ~68 kDa)

Antibody: E2A.E12 (V-18), Santa Cruz Biotechnology (sc-349)
Rabbit polyclonal, epitope mapping at the C-terminus of E2A.E12 of human origin
Web: http://www.scbt.com/datasheet-349-e2a-e12-v-18-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

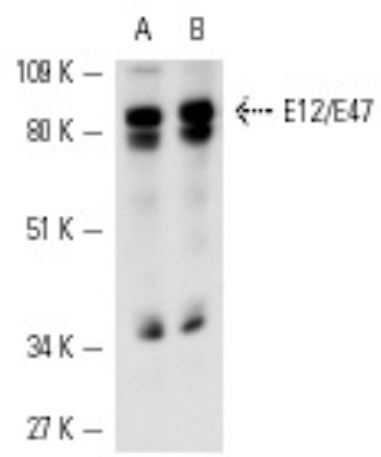

Figure Legend: Western blot analysis of E12/E47 expression in Jurkat (A) and K562 (B) whole cell lysates.

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.

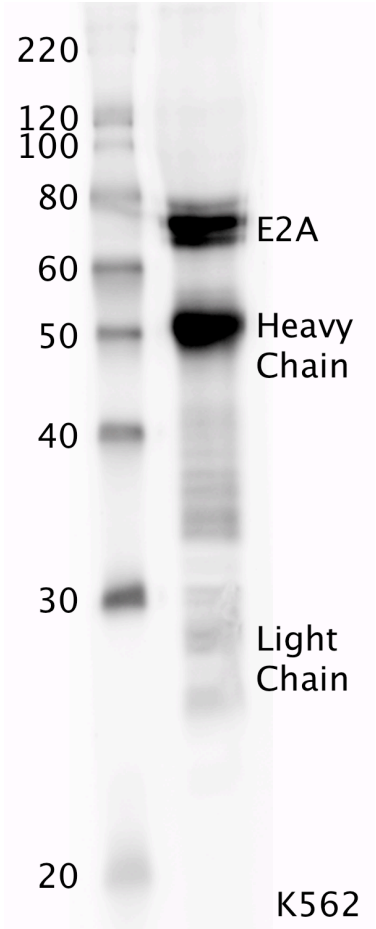

Figure Legend: TCF3 immunoblot: IP-western with sc-349 E2A.E12 antibody in whole cell lysate of K562. Heavy chain and light chain of IgG are indicated, and TCF3 band is indicated at ~70 kDa.

Validation 2: In progress