

ENCODE Antibody Validation Documentation

Transcription factor: B-cell CLL/lymphoma 3 (GeneID 602)

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Transcription factor: BCL3 (GeneID 602; ~47 kDa)

Antibody: BCL3 (C-14), Santa Cruz Biotechnology (sc-185)
Rabbit polyclonal, epitope mapping at the C-terminus of BCL3 of human origin
Web: <http://www.scbt.com/datasheet-185-bcl-3-c-14-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

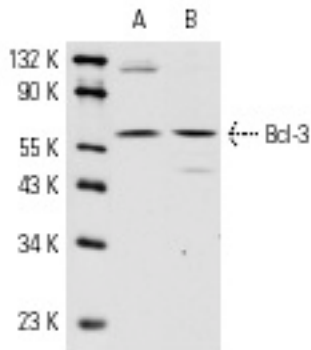


Figure Legend: Western blot analysis of BCL3 expression in NAMALWA (A) and WEHI-3 (B) whole cell lysates.

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 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).

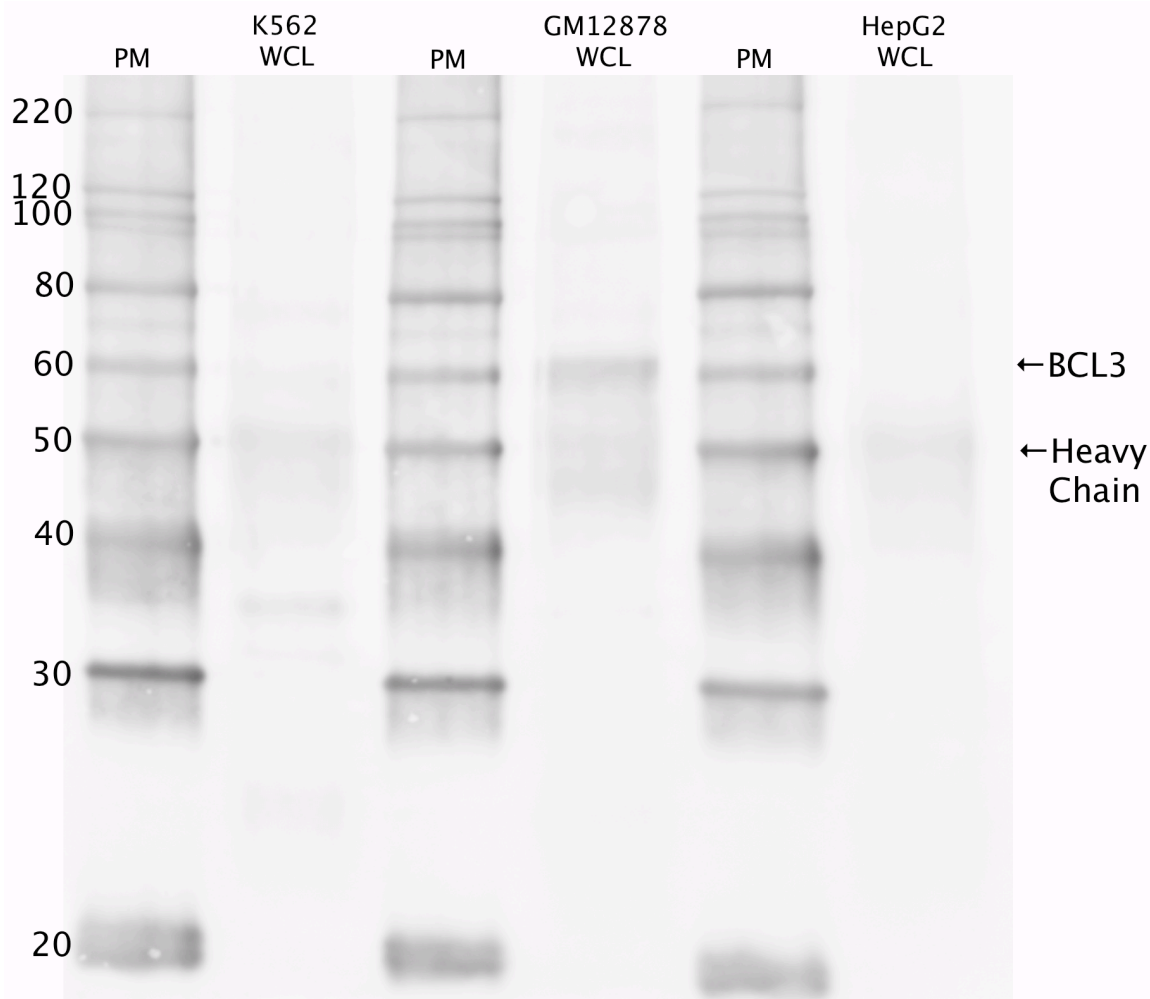


Figure Legend: BCL3 immunoblot: IP-western with sc-185 BCL3 antibody in whole cell lysates (WCL) of K562, GM12878, and HepG2. Heavy chain of IgG is indicated, and BCL3 band is indicated at ~60 kDa.

Validation 2: In progress