

**ENCODE Antibody Validation Documentation**  
**Transcription factor: BCL2-associated transcription factor 1**  
**(GeneID 9774)**

**From: Myers Lab, HudsonAlpha Institute for Biotechnology**

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**Transcription factor:** BCLAF1 (GeneID 9774; ~106 kDa)

**Antibody:** BCLAF1 (M33-P5B11), Santa Cruz Biotechnology (sc-101388)

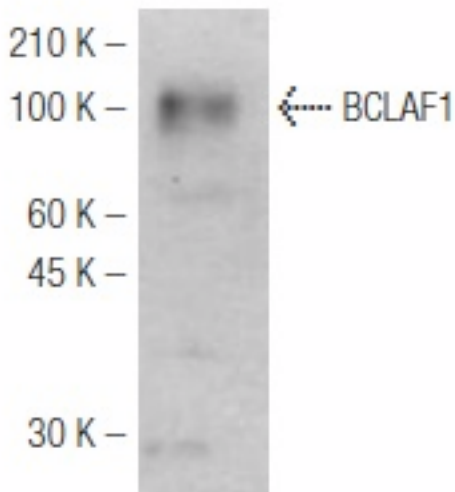
Mouse monoclonal, raised against synthetic peptide corresponding to amino acids 900-909 of BCLAF1 of human origin

Web: <http://www.scbt.com/datasheet-101388-bclaf1-m33-p5b11-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

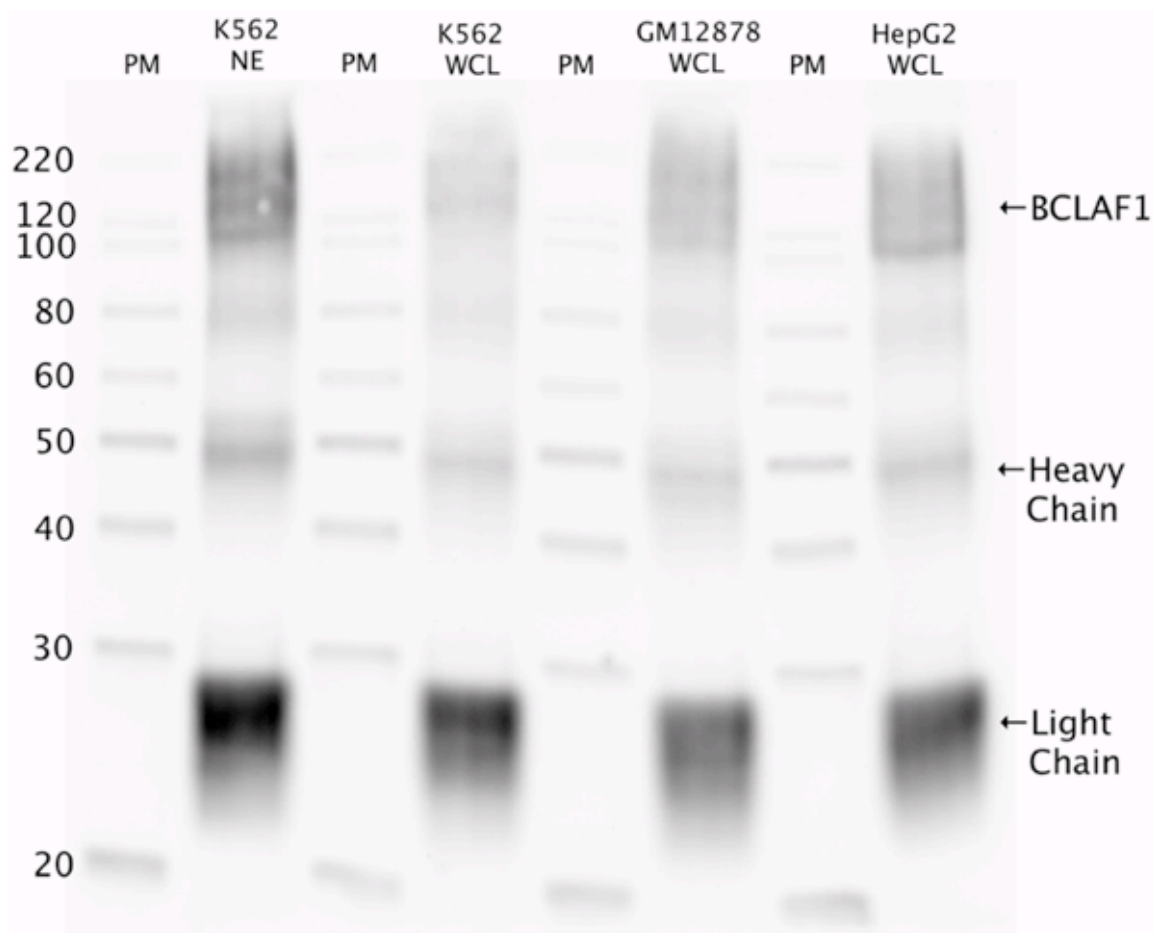


**Figure Legend:** Western blot analysis of BCLAF1 expression in HeLa whole cell lysate.

## 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 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:** BCLAF1 immunoblot: IP-western with sc-101388 BCLAF1 antibody in nuclear lysate (NE) or whole cell lysates (WCL) of K562, GM12878, and HepG2. Heavy chain and light chain of IgG are indicated, and BCLAF1 band and smear are indicated at ~106 kDa and higher.

**Validation 2: In progress**