

ENCODE Antibody Validation Documentation
Transcription factor: Early B-cell factor 1 (GeneID 1879)

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Transcription factor: EBF (GeneID 1879; ~64 kDa)

Antibody: EBF (C-8), Santa Cruz Biotechnology (sc-137065)
Mouse monoclonal, raised against amino acids 1-300 mapping at the N-terminus of EBF of human origin
Web: <http://www.scbt.com/datasheet-137065-ebf-c-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

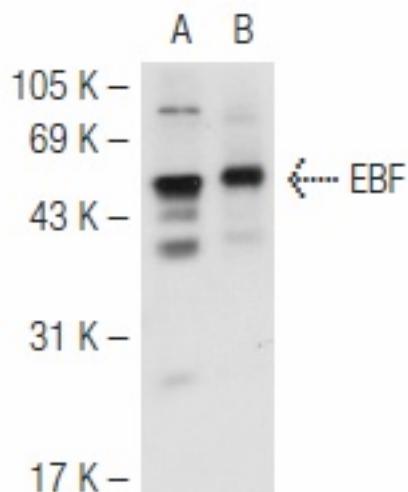


Figure Legend: Western blot analysis of EBF expression in Ramos (A) and IMR-32 (B) whole cell lysates.

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).

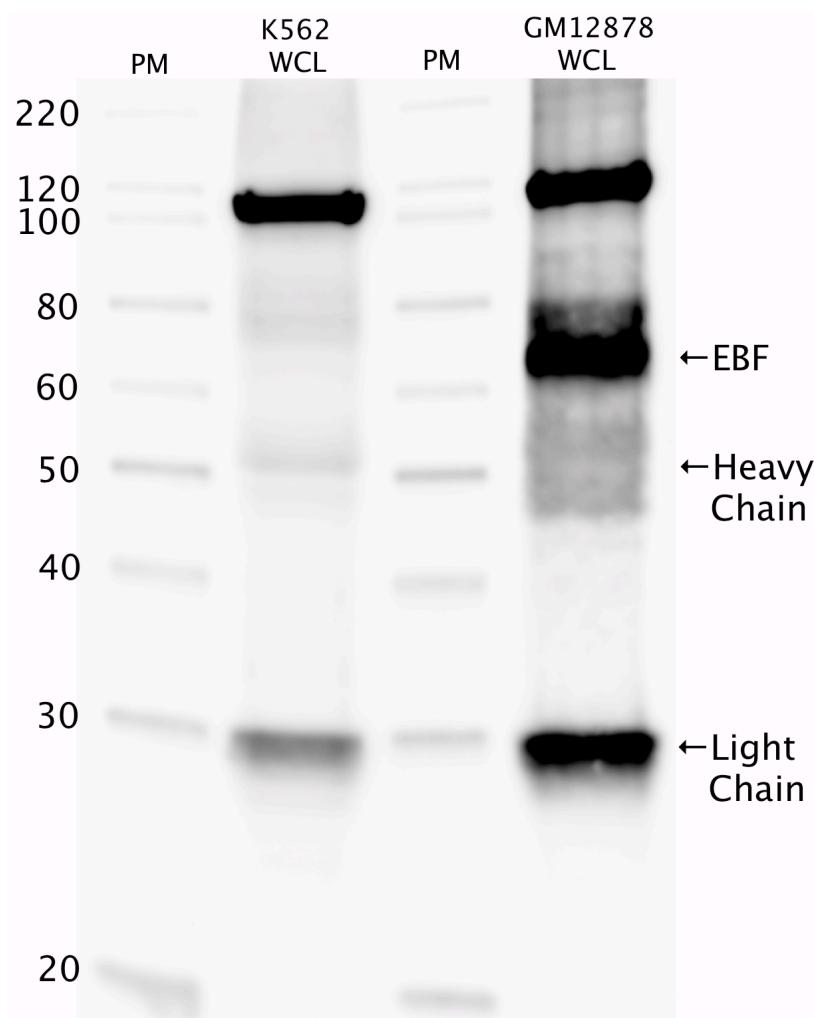


Figure Legend: EBF immunoblot: IP-western with sc-137065 EBF antibody in whole cell lysates (WCL) of K562 and GM12878. Heavy chain and light chain of IgG are indicated, and EBF band is indicated at ~64 kDa.

Validation 2: In progress