

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

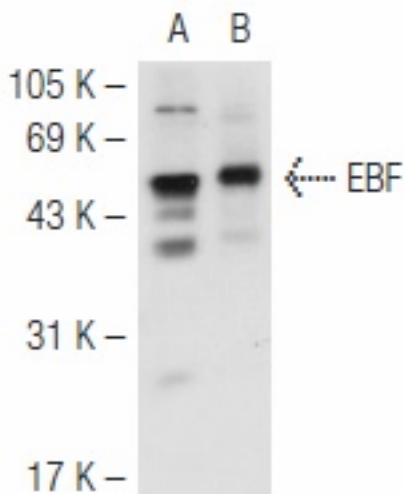


Figure Legend: Western blot analysis of EBF expression in Ramos (A) and IMR-32 (B) whole cell lysates.

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).

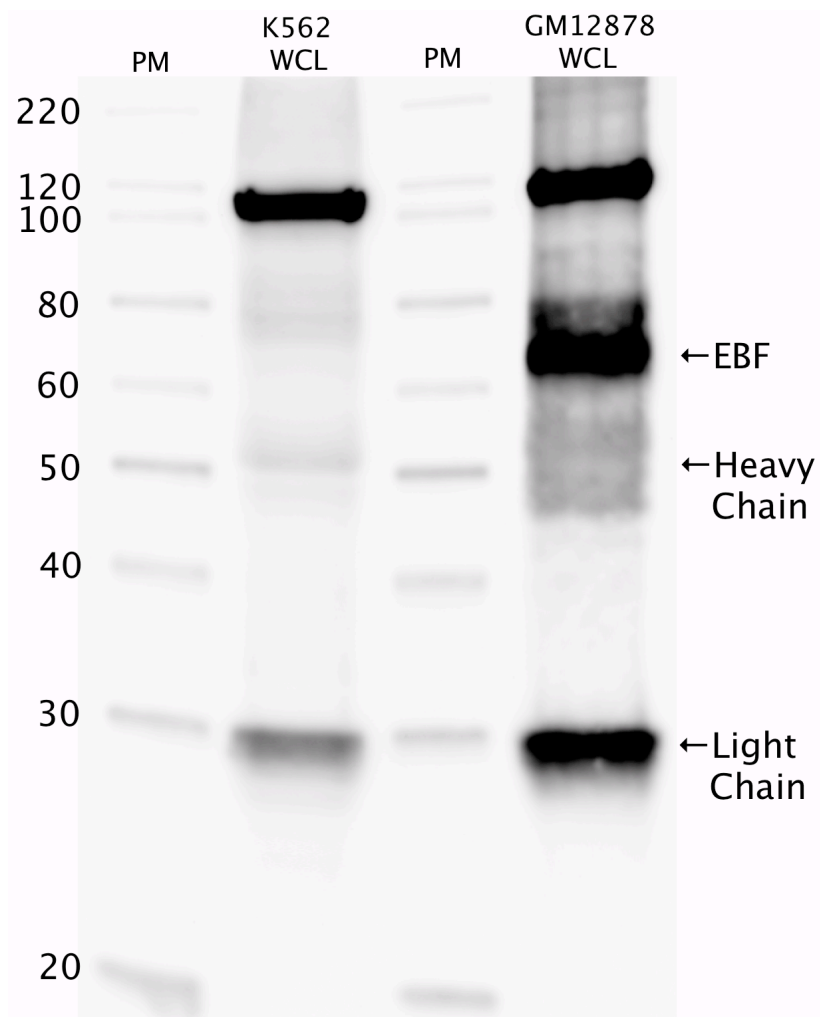


Figure Legend: EBF immunoblot: IP-western with sc-137065 EBF antibody in whole cell lysates (WCL) of K562 and GM12878. Heavy chain and light chain of IgG are indicated, and EBF band is indicated at ~64 kDa.

Validation 2: Mass Spectrometry Analysis

ENCODE data standards recognizes various methodologies for secondary validation of antibodies. Among these methodologies is immunoprecipitation followed by mass spectrometry analysis. Briefly, GM12878 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. Gel was stained with Coomassie Blue in order to visualize marker bands. A gel fragment corresponding to the band indicated above in the western blot image was excised and sent to the University of Alabama at Birmingham Cancer Center Mass Spectrometry/Proteomics Shared Facility. There the sample was run on an LTQ XL Linear Ion Trap Mass Spectrometer with alternating collision-induced dissociation and electron-transfer dissociation. Peptides were identified using MASCOT (Matrix Science), with probability based matching at $p < 0.05$. Subsequent analysis was performed in Scaffold (Proteome Software, Inc.) at 0.0% protein FDR and 0.0% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM2 PE=1 SV=4 KPYM_HUMAN

Tyrosine-protein phosphatase non-receptor type 6 OS=Homo sapiens GN=PTPN6 PE=1 SV=1 PTN6_HUMAN

Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 K2C1_HUMAN

Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6 K1C10_HUMAN

Bifunctional purine biosynthesis protein PURH OS=Homo sapiens GN=ATIC PE=1 SV=3 PUR9_HUMAN

T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4 TCPG_HUMAN

T-complex protein 1 subunit alpha OS=Homo sapiens GN=TCP1 PE=1 SV=1 TCPA_HUMAN

60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2 CH60_HUMAN

Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2 K22E_HUMAN

Non-POU domain-containing octamer-binding protein OS=Homo sapiens GN=NONO PE=1 SV=4 NONO_HUMAN

T-complex protein 1 subunit epsilon OS=Homo sapiens GN=CCT5 PE=1 SV=1 TCPE_HUMAN

Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1 DDX5_HUMAN

Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK PE=1 SV=1 HNRPK_HUMAN

T-complex protein 1 subunit eta OS=Homo sapiens GN=CCT7 PE=1 SV=2 T2CPH_HUMAN

General vesicular transport factor p115 OS=Homo sapiens GN=USO1 PE=1 SV=2 USO1_HUMAN

Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3 K1C9_HUMAN

T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3 TCPZ_HUMAN

WD repeat-containing protein 1 OS=Homo sapiens GN=WDR1 PE=1 SV=4 WDR1_HUMAN

26S proteasome non-ATPase regulatory subunit 3 OS=Homo sapiens GN=PSMD3 PE=1 SV=2 PSMD3_HUMAN

Eukaryotic translation initiation factor 3 subunit D OS=Homo sapiens GN=EIF3D PE=1 SV=1 EIF3D_HUMAN

Eukaryotic translation initiation factor 3 subunit L OS=Homo sapiens GN=EIF3L PE=1 SV=1 EIF3L_HUMAN

Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2 ENOA_HUMAN

Guanine nucleotide-binding protein-like 3 OS=Homo sapiens GN=GNL3 PE=1 SV=2 GNL3_HUMAN

Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4 HS90B_HUMAN

Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1 TBA1B_HUMAN

Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 ACTB_HUMAN (+1)

Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 ALBU_HUMAN

Transcription factor COE1 OS=Homo sapiens GN=EBF1 PE=1 SV=2 COE1_HUMAN

Coatomer subunit delta OS=Homo sapiens GN=ARCN1 PE=1 SV=1 COPD_HUMAN

T-complex protein 1 subunit delta OS=Homo sapiens GN=CCT4 PE=1 SV=4 TCPD_HUMAN