

ENCODE Antibody Validation Documentation
Transcription factor: Activating transcription factor 3 (GeneID 467)

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Transcription factor: ATF3 (GeneID 467; ~20 kDa)

Antibody: ATF3 (C-19), Santa Cruz Biotechnology (sc-188)
Rabbit polyclonal, epitope mapping at C-terminus of ATF3 of human origin
Web: <http://www.scbt.com/datasheet-188-atf-3-c-19-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

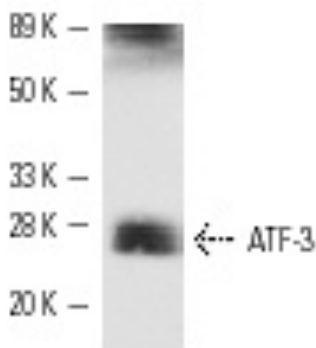


Figure Legend: Western blot analysis of ATF3 expression in RAW 264.7 whole cell lysate.

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).

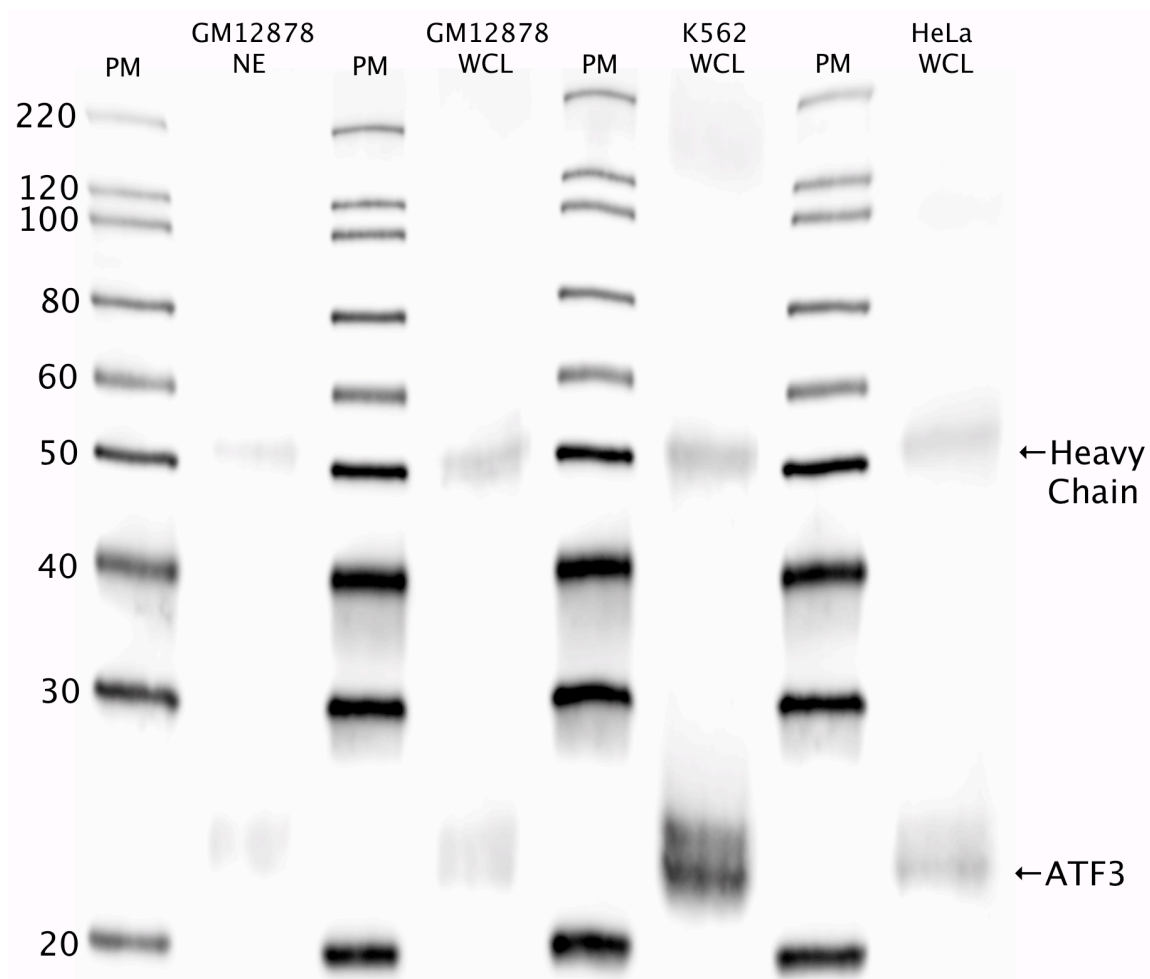


Figure Legend: ATF3 immunoblot: IP-western with sc-188 ATF3 antibody in nuclear lysate (NE) or whole cell lysates (WCL) of GM12878, K562, and HeLa. Heavy chain of IgG is indicated, and ATF3 band is indicated at ~23 kDa.

Validation 2: In progress