ENCODE Antibody Validation Documentation
Transcription factor: FOS-like antigen 2 (GeneID 2355)

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Transcription factor: FOSL2 (GeneID 2355; ~35 kDa)

Antibody: Fra-2 (Q-20), Santa Cruz Biotechnology (sc-604)
Rabbit polyclonal, epitope mapping at the N-terminus of Fra-2 of human origin
Web: http://www.scbt.com/datasheet-604-fra-2-q-20-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

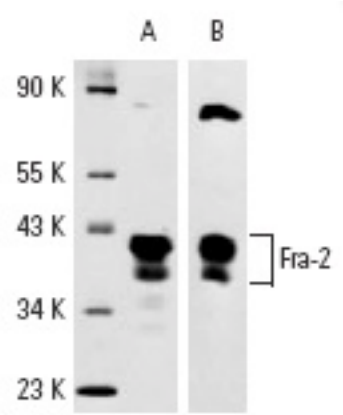

Figure Legend: Western blot analysis of Fra-2 expression in NIH/3T3 nuclear extracts (A, B). Fra-2 (Q-20) antibody (sc-604) is tested in lane (B).

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 using a Bio-Rad Trans-Blot Electrophoretic Transfer system. Standard western blot protocol was used to probe the membrane with the primary antibody (same antibody as used for IP), and an HRP-conjugated secondary antibody and SuperSignal West Femto solution (Thermo Scientific) were used to detect the immunoprecipitated proteins.

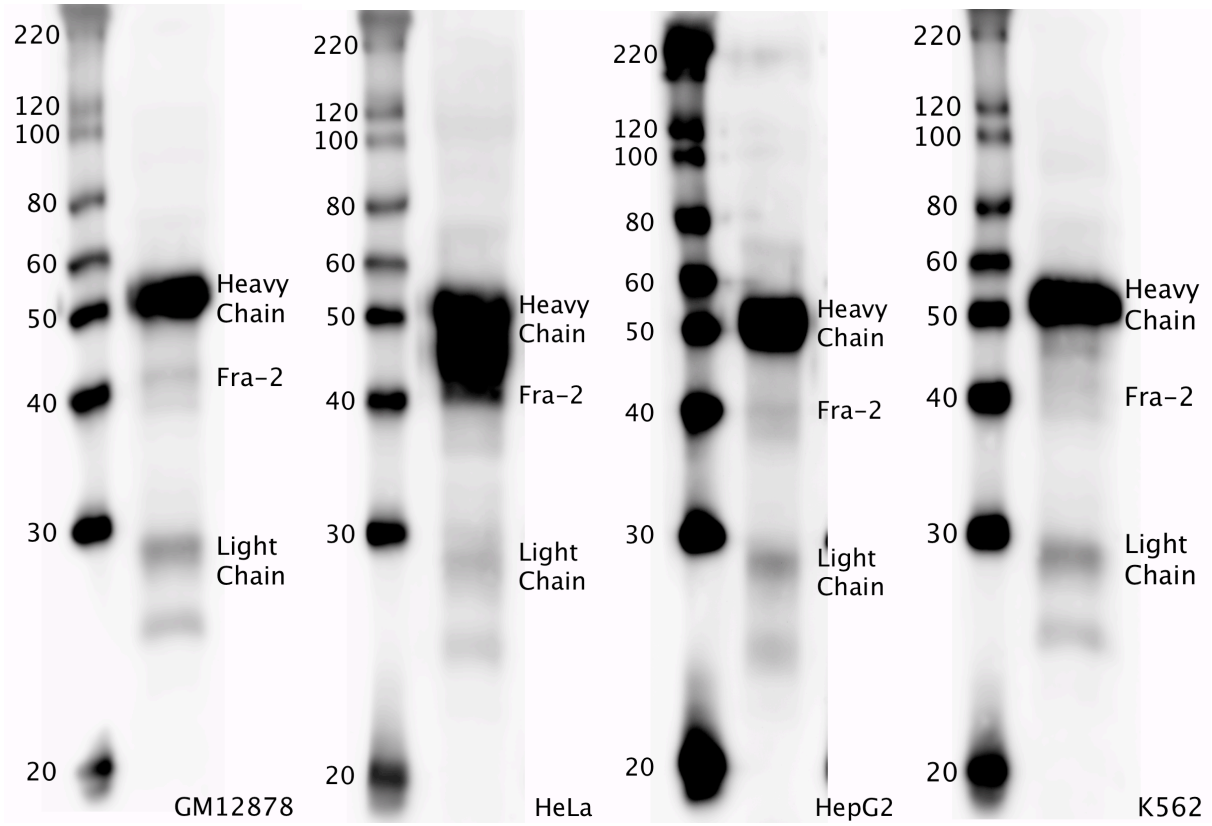

Figure Legend: FOSL2 immunoblot: IP-western with sc-604 FOSL2 antibody in whole cell lysates of, left to right: GM12878, HeLa, HepG2, and K562. Heavy chain and light chain of IgG are indicated, and Fra-2 band is indicated at ~40 kDa.

Validation 2: In progress