ENCODE Antibody Validation Documentation
Transcription factor: GA binding protein transcription factor, alpha subunit 60kDa (GeneID 2551)

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Transcription factor: GABPA (GeneID 2551; ~51 kDa)

Antibody: GABP-α (G-1), Santa Cruz Biotechnology (sc-28312)
Mouse monoclonal, raised against amino acids 1-180 of GABP-α of human origin
Web: http://www.scbt.com/datasheet-28312-gabp-alpha-g-1-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

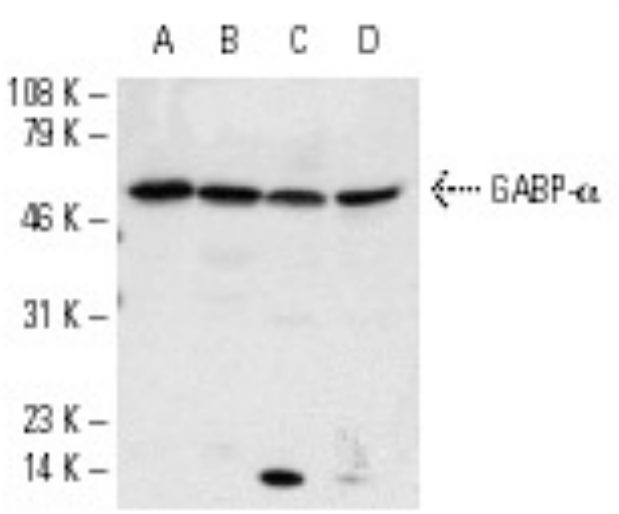

Figure Legend: Western blot analysis of GABP-α expression in HeLa (A), A-431 (B), NIH/3T3 (C) and 3611-RF (D) nuclear extracts.

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.

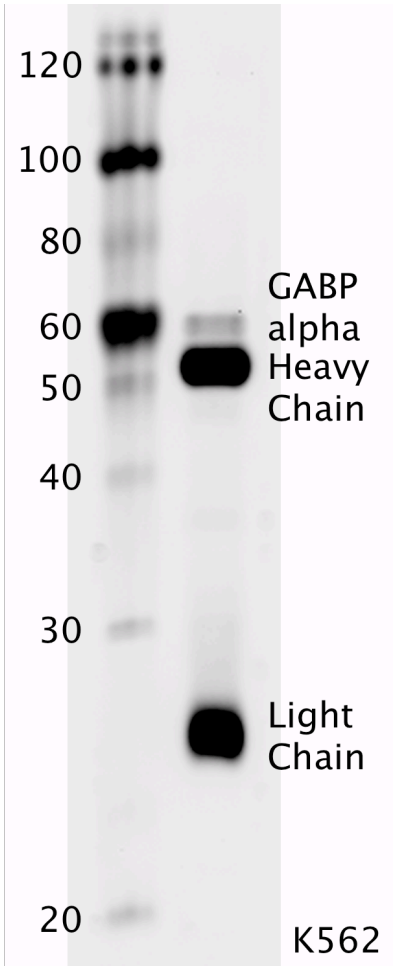

Figure Legend: GABPA immunoblot: IP-western with sc-28312 GABP-α antibody in whole cell lysate of K562. Heavy chain and light chain of IgG are indicated, and GABPA band is indicated at ~60 kDa.

Validation 2: In progress