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.
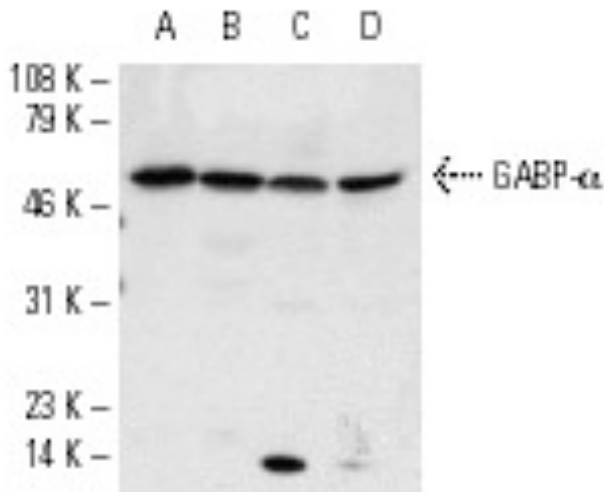
a. Vendor immunoblot analysis

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.
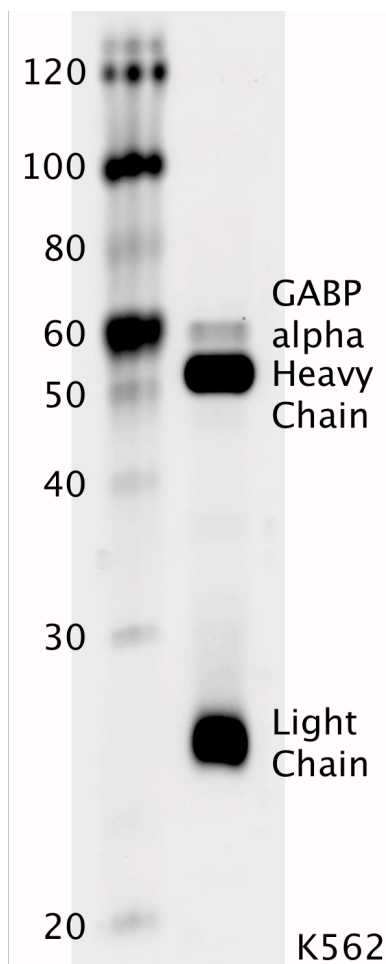

Figure Legend: GABPA immunoblot: IP-western with sc-28312 GABP-α antibody in whole cell lysate of K562. Heavy chain and light chain of IgG are indicated, and GABPA band is indicated at ~60 kDa.

Validation 2: 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 Coomasie 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

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 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

Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4   MYH9_HUMAN

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

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

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

Glucose-6-phosphate isomerase OS=Homo sapiens GN=GPI PE=1 SV=4 G6PI_HUMAN

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

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

Coronin-1A OS=Homo sapiens GN=CORO1A PE=1 SV=4   COR1A_HUMAN

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

T-complex protein 1 subunit theta OS=Homo sapiens GN=CCT8 PE=1 SV=4   TCPQ_HUMAN

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

Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2   IGHA1_HUMAN

T-complex protein 1 subunit beta OS=Homo sapiens GN=CCT2 PE=1 SV=4   TCPB_HUMAN

Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4   VIME_HUMAN

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

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

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

Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3   PDIA1_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

Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15 OS=Homo sapiens GN=DHX15 PE=1 SV=2   DHX15_HUMAN

Polypyrimidine tract-binding protein 1 OS=Homo sapiens GN=PTBP1 PE=1 SV=1   PTBP1_HUMAN

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

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

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

Adenylyl cyclase-associated protein 1 OS=Homo sapiens GN=CAP1 PE=1 SV=4   CAP1_HUMAN

ATP-dependent RNA helicase DDX3X OS=Homo sapiens GN=DDX3X PE=1 SV=3 DDX3X_HUMAN

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

GA-binding protein alpha chain OS=Homo sapiens GN=GABPA PE=1 SV=1   GABPA_HUMAN

Serine/threonine-protein kinase PAK 2 OS=Homo sapiens GN=PAK2 PE=1 SV=3   PAK2_HUMAN