

**ENCODE Antibody Validation Documentation**  
**Transcription factor: hepatocyte nuclear factor 4, alpha (GeneID 3172)**

**From: Myers Lab, HudsonAlpha Institute for Biotechnology**

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**Transcription factor:** HNF4A (GeneID 3172; ~53 kDa)

**Antibody:** HNF-4 $\alpha$  (H-171), Santa Cruz Biotechnology (sc-8987)

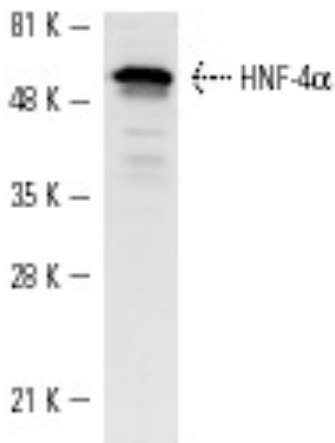
Rabbit polyclonal, epitope corresponding to amino acids 295-465 mapping at C-terminus of HNF-4 $\alpha$  of human origin

Web: <http://www.scbt.com/datasheet-8987-hnf-4alpha-h-171-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

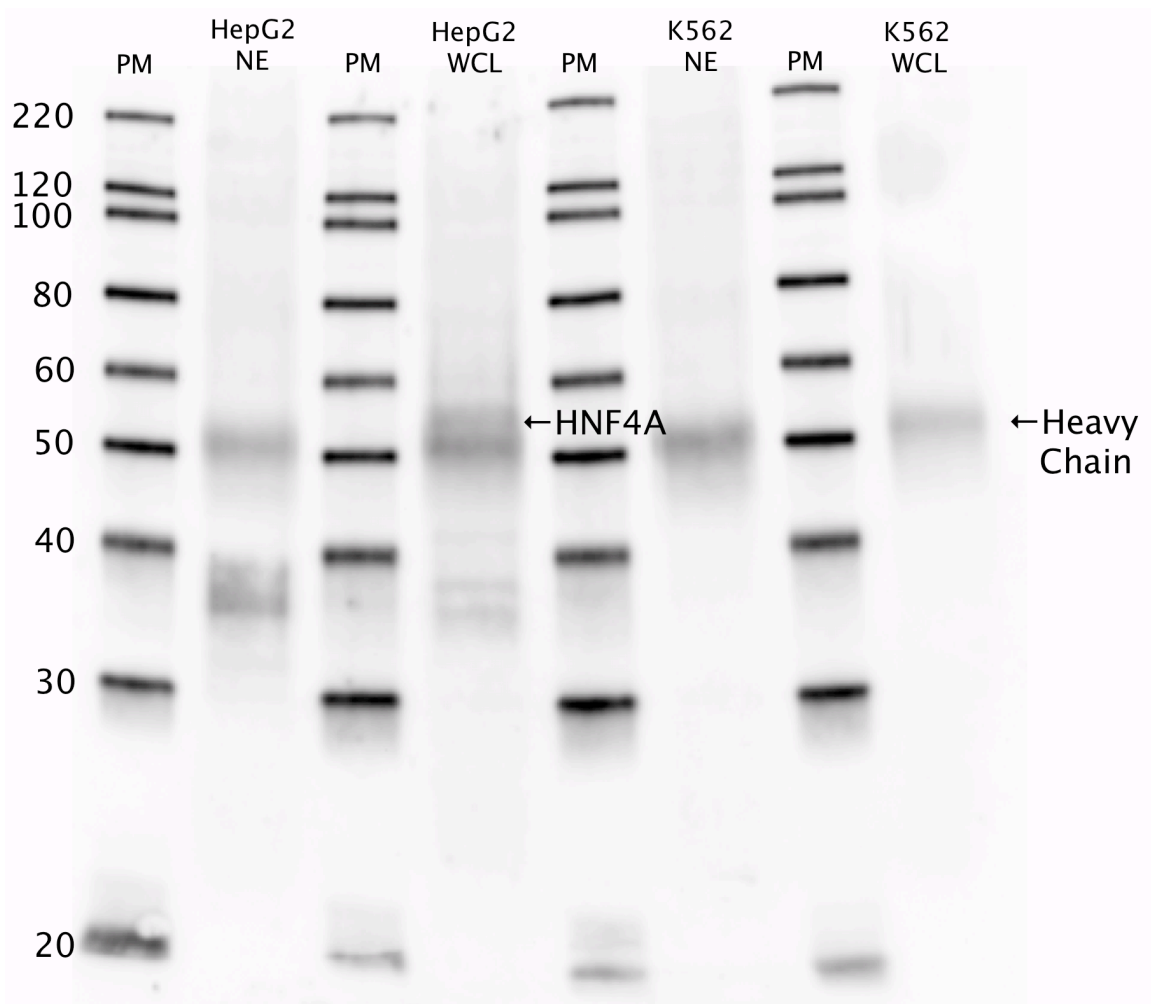


**Figure Legend:** Western blot analysis of HNF-4 $\alpha$  expression in HepG2 whole cell lysate.

## b. Myers Lab immunoblot analysis

### Western blot protocol

Whole cell lysates or nuclear extracts 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:** HNF4A immunoblot: IP-western with sc-8987 HNF-4 $\alpha$  antibody in nuclear extract (NE) or whole cell lysates (WCL) of HepG2 and K562. Heavy chain of IgG is indicated, and HNF4A band is indicated at ~55 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, HepG2 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 at ~55 kDa and a second fragment at ~38 kDa were excised and sent to the University of Alabama at Birmingham Cancer Center Mass Spectrometry/Proteomics Shared Facility. There the samples were 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 1.7% peptide FDR. As per ENCODE data standards, all Scaffold results are listed below, including common contaminants. Target protein is highlighted in bold font.

Upper band (~55 kDa):

Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C\_HUMAN P68371 (+2)

ATP synthase subunit alpha, mitochondrial n=3 Tax=Homininae RepID=ATPA\_HUMAN P25705

cDNA FLJ54023, highly similar to Heat shock protein HSP 90-beta n=1 Tax=Homo sapiens RepID=B4DMA2\_HUMAN B4DMA2 (+1)

Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA\_HUMAN P06733

ATP synthase subunit beta, mitochondrial n=1 Tax=Homo sapiens RepID=ATPB\_HUMAN P06576

cDNA FLJ32131 fis, clone PEBLM2000267, highly similar to Tubulin alpha-ubiquitous chain n=1 Tax=Homo sapiens RepID=B3KPS3\_HUMAN B3KPS3 (+2)

Aspartyl-tRNA synthetase, cytoplasmic n=4 Tax=Homo sapiens RepID=SYDC\_HUMAN P14868

cDNA FLJ16143 fis, clone BRAMY2038516, highly similar to Protein disulfide-isomerase A6 (EC 5.3.4.1) n=1 Tax=Homo sapiens RepID=B3KY95\_HUMAN B3KY95 (+4)

RuvB-like 1 (Fragment) n=1 Tax=Homo sapiens RepID=B5BUB1\_HUMAN B5BUB1 (+1)

Heterogeneous nuclear ribonucleoprotein H, N-terminally processed n=2 Tax=Homo sapiens RepID=HNRH1\_HUMAN P31943 (+1)

Eukaryotic peptide chain release factor subunit 1 n=7 Tax=Eutheria RepID=ERF1\_HUMAN P62495

T-complex protein 1 subunit delta n=1 Tax=Homo sapiens RepID=A8K3C3\_HUMANA8K3C3 (+1)

Tubulin beta chain n=12 Tax=Amniota RepID=TBB5\_HUMAN P07437

Pyruvate kinase isozymes M1/M2 n=2 Tax=Homininae RepID=KPYM\_HUMAN P14618

PIG48 n=1 Tax=Homo sapiens RepID=Q2TU64\_HUMAN Q2TU64

cDNA FLJ78120, highly similar to Homo sapiens eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa (EIF2S3), mRNA n=1 Tax=Homo sapiens RepID=A8K2Y2\_HUMAN A8K2Y2 (+2)

Retinoblastoma binding protein 7 n=2 Tax=Catarrhini RepID=B0R0W4\_HUMAN B0R0W4 (+4)

cDNA FLJ60399, highly similar to ATP-dependent RNA helicase DDX3X (EC 3.6.1.-) n=1 Tax=Homo sapiens RepID=B4E3E8\_HUMAN B4E3E8 (+2)

T-complex protein 1 subunit alpha n=2 Tax=Homininae RepID=TCPA\_HUMAN P17987

Probable ATP-dependent RNA helicase DDX6 n=2 Tax=Homo sapiens RepID=DDX6\_HUMAN P26196

**Hepatocyte nuclear factor 4-alpha n=1 Tax=Homo sapiens RepID=HNF4A\_HUMAN P41235 (+4)**

Spliceosome RNA helicase BAT1 n=7 Tax=Eutheria RepID=UAP56\_HUMAN Q13838 (+1)

Tubulin beta-2A chain n=4 Tax=Euarchontoglires RepID=TBB2A\_HUMAN Q13885

Dha kinase/FMN cyclase splice variant n=1 Tax=Homo sapiens RepID=Q2L9C1\_HUMAN Q2L9C1 (+1)

Lower band (~38 kDa):

L-lactate dehydrogenase A chain n=3 Tax=Homo sapiens RepID=LDHA\_HUMAN P00338

cDNA FLJ54023, highly similar to Heat shock protein HSP 90-beta n=1 Tax=Homo sapiens RepID=B4DMA2\_HUMAN B4DMA2 (+1)

L-lactate dehydrogenase B chain n=4 Tax=Catarrhini RepID=LDHB\_HUMAN P07195

**Hepatocyte nuclear factor 4-alpha n=1 Tax=Homo sapiens RepID=HNF4A\_HUMAN P41235 (+4)**

Prohibitin-2 n=3 Tax=Euarchontoglires RepID=PHB2\_HUMAN Q99623 (+1)

cDNA FLJ75549, highly similar to Homo sapiens ribosomal protein, large, P0 (RPLP0), transcript variant 1, mRNA n=1 Tax=Homo sapiens RepID=A8K4Z4\_HUMAN A8K4Z4 (+3)

cDNA FLJ52842, highly similar to Actin, cytoplasmic 1 n=1 Tax=Homo sapiens RepID=B4E335\_HUMAN B4E335 (+7)

Alpha-enolase n=1 Tax=Homo sapiens RepID=ENOA\_HUMAN P06733

Tubulin beta-2C chain n=3 Tax=Eutheria RepID=TBB2C\_HUMAN P68371 (+2)

cDNA FLJ32131 fis, clone PEBLM2000267, highly similar to Tubulin alpha-ubiquitous chain n=1 Tax=Homo sapiens RepID=B3KPS3\_HUMAN B3KPS3 (+2)

Glyceraldehyde-3-phosphate dehydrogenase n=1 Tax=Homo sapiens RepID=G3P\_HUMAN P04406 (+1)

60S ribosomal protein L5 n=3 Tax=Homo sapiens RepID=RL5\_HUMAN P46777

cDNA FLJ57726, highly similar to Heterogeneous nuclear ribonucleoprotein H3 n=1 Tax=Homo sapiens RepID=B4DHY1\_HUMAN B4DHY1 (+3)

cDNA FLJ56307, highly similar to Ubiquitin thioesterase protein OTUB1 (EC 3.4.-.-) n=1 Tax=Homo sapiens RepID=B4DPD5\_HUMAN B4DPD5 (+4)

Putative uncharacterized protein GNB2 n=3 Tax=Euarchontoglires RepID=C9JXA5\_HUMAN C9JXA5 (+2)

Heterogeneous nuclear ribonucleoprotein A1 n=1 Tax=Homo sapiens RepID=ROA1\_HUMAN P09651 (+3)

Pyruvate kinase isozymes M1/M2 n=2 Tax=Homininae RepID=KPYM\_HUMAN P14618

rRNA 2'-O-methyltransferase fibrillarin n=1 Tax=Homo sapiens RepID=FBRL\_HUMAN P22087

ATP synthase subunit alpha, mitochondrial n=3 Tax=Homininae RepID=ATPA\_HUMAN P25705

60S ribosomal protein L4 n=1 Tax=Homo sapiens RepID=RL4\_HUMAN P36578

Poly(rC)-binding protein 1 n=7 Tax=Eutheria RepID=PCBP1\_HUMAN Q15365

Pyrraline-5-carboxylate reductase 2 n=1 Tax=Homo sapiens RepID=P5CR2\_HUMAN Q96C36

cDNA FLJ76127, highly similar to Homo sapiens replication factor C (activator 1) 5, 36.5kDa (RFC5), transcript variant 1, mRNA n=1 Tax=Homo sapiens RepID=A8K3S0\_HUMAN A8K3S0 (+7)

Elongation factor 1-alpha n=1 Tax=Homo sapiens RepID=A8K9C4\_HUMAN A8K9C4 (+9)

60S ribosomal protein L6 n=1 Tax=Homo sapiens RepID=B2R4K7\_HUMAN B2R4K7 (+4)

Malate dehydrogenase n=2 Tax=Homininae RepID=B4DUN2\_HUMAN B4DUN2 (+3)

Putative uncharacterized protein HNRNPA2B1 n=1 Tax=Homo sapiens RepID=B8ZZ37\_HUMAN B8ZZ37 (+1)

Tubulin beta chain n=12 Tax=Amniota RepID=TBB5\_HUMAN P07437

Proliferating cell nuclear antigen n=3 Tax=Catarrhini RepID=PCNA\_HUMAN P12004 (+2)

Coproporphyrinogen-III oxidase, mitochondrial n=1 Tax=Homo sapiens RepID=HEM6\_HUMAN P36551

Aldo-keto reductase family 1 member C3 n=1 Tax=Homo sapiens RepID=AK1C3\_HUMAN P42330

Translocase of inner mitochondrial membrane 50 homolog (S. cerevisiae) n=1 Tax=Homo sapiens RepID=Q0VAB1\_HUMAN Q0VAB1 (+2)

Endothelial monocyte-activating polypeptide 2 n=2 Tax=Homo sapiens RepID=AIMP1\_HUMAN Q12904

Heat shock protein 75 kDa, mitochondrial n=2 Tax=Homo sapiens RepID=TRAP1\_HUMAN Q12931 (+1)