

**ENCODE Antibody Validation Documentation**  
**Transcription factor: SIN3 homolog A, transcription regulator**  
**(GeneID 25942)**

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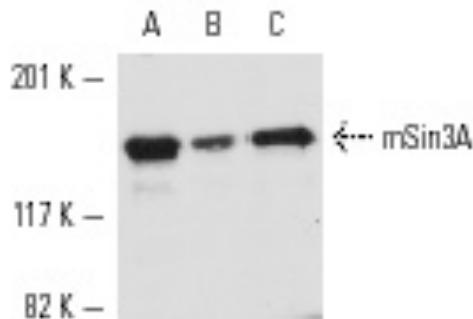
**Transcription factor:** SIN3A (GeneID 25942; ~145 kDa)

**Antibody:** mSin3A (K-20), Santa Cruz Biotechnology (sc-994)  
Rabbit polyclonal, epitope mapping at the N-terminus of mSin3A of mouse origin  
Web: <http://www.scbt.com/datasheet-994-msin3a-k-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**

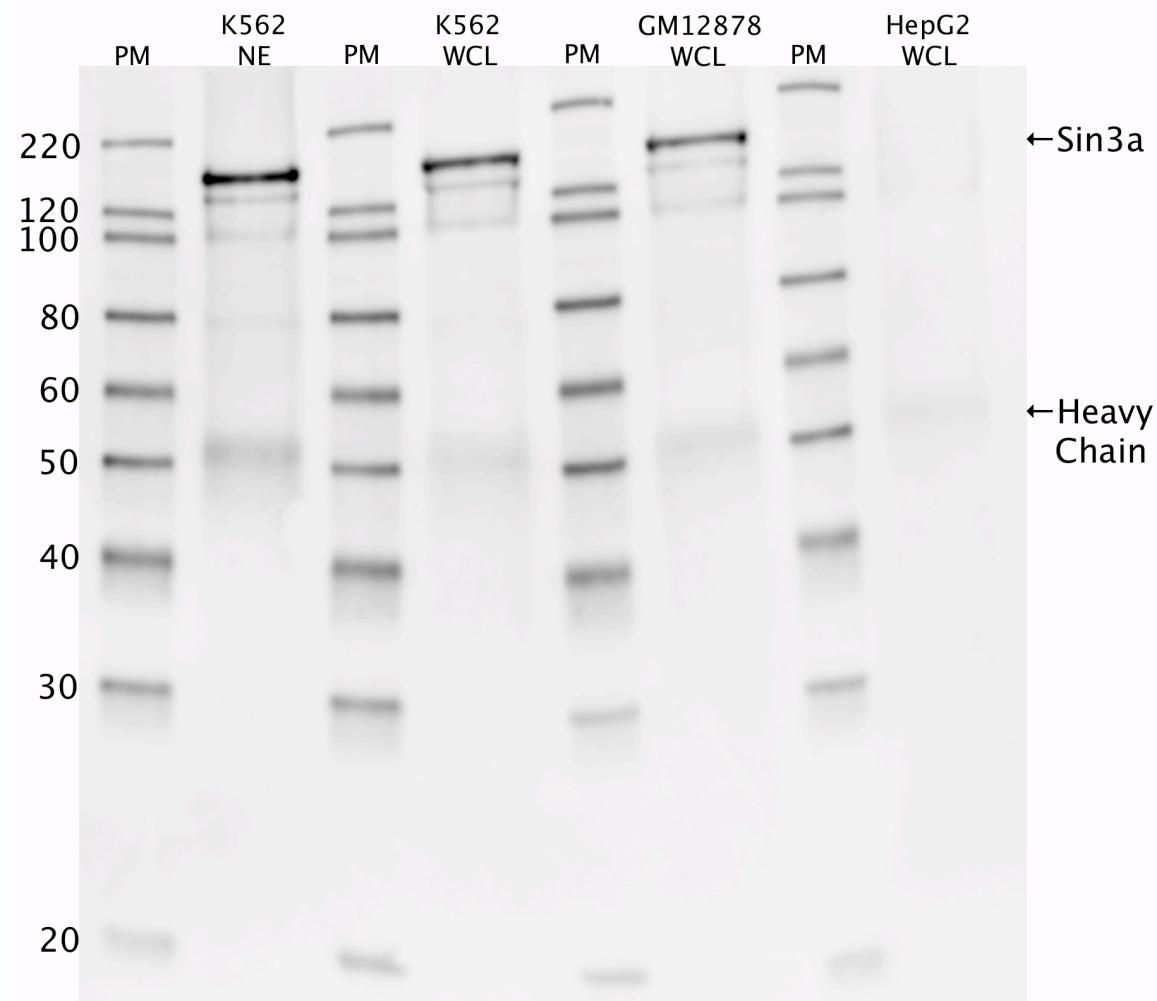


**Figure Legend:** Western blot analysis of mSin3A expression in HeLa (A), Jurkat (B) and K562 (C) whole cell lysates.

## 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:** SIN3A immunoblot: IP-western with sc-994 mSIN3A antibody in nuclear extract (NE) and whole cell lysate (WCL) of K562, and whole cell lysates of GM12878 and HepG2. Heavy chain of IgG is indicated, and SIN3A band is indicated at ~150 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, K562 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.

Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=5 SYEP\_HUMAN

Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens GN=LRPPRC PE=1 SV=3 LPPRC\_HUMAN

ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4 DHX9\_HUMAN

Splicing factor 3B subunit 1 OS=Homo sapiens GN=SF3B1 PE=1 SV=3 SF3B1\_HUMAN

Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3 FAS\_HUMAN

Isoleucyl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=IARS PE=1 SV=2 SYIC\_HUMAN

Valyl-tRNA synthetase OS=Homo sapiens GN=VARS PE=1 SV=4 SYVC\_HUMAN

Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5 CLH1\_HUMAN

DNA-dependent protein kinase catalytic subunit OS=Homo sapiens GN=PRKDC PE=1 SV=3 PRKDC\_HUMAN

Leucyl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=LARS PE=1 SV=2 SYLC\_HUMAN

Hypoxia up-regulated protein 1 OS=Homo sapiens GN=HYOU1 PE=1 SV=1 HYOU1\_HUMAN

Exportin-5 OS=Homo sapiens GN=XPO5 PE=1 SV=1 XPO5\_HUMAN

**Paired amphipathic helix protein Sin3a OS=Homo sapiens GN=SIN3A PE=1 SV=2 SIN3A\_HUMAN**

Coatomer subunit alpha OS=Homo sapiens GN=COPA PE=1 SV=2 COPA\_HUMAN

Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 K2C1\_HUMAN

Protein diaphanous homolog 1 OS=Homo sapiens GN=DIAPH1 PE=1 SV=2 DIAP1\_HUMAN

Eukaryotic translation initiation factor 3 subunit A OS=Homo sapiens GN=EIF3A PE=1 SV=1 EIF3A\_HUMAN

Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3 K1C9\_HUMAN

Myb-binding protein 1A OS=Homo sapiens GN=MYBBP1A PE=1 SV=2 MBB1A\_HUMAN

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

Ras GTPase-activating-like protein IQGAP1 OS=Homo sapiens GN=IQGAP1 PE=1 SV=1 IQGA1\_HUMAN

Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16 OS=Homo sapiens GN=DHX38 PE=1 SV=2 PRP16\_HUMAN