

ENCODE Antibody Validation Documentation

Transcription factor: Estrogen Receptor Alpha (GeneID 2099)

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Transcription factor: ESR1 (GeneID 2099; ~66 kDa)

Antibody: ER α (HC-20), Santa Cruz Biotechnology (sc-543)

Rabbit polyclonal, epitope mapping at the C-terminus of ER α of human origin

Web: <http://www.scbt.com/datasheet-543-eralpha-hc-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

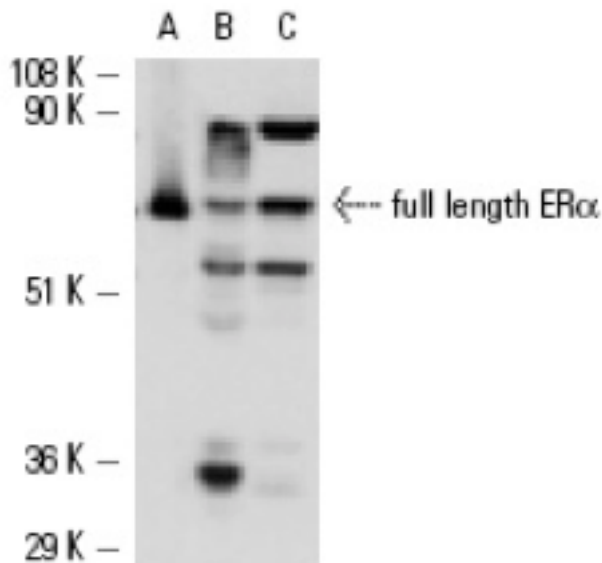


Figure Legend: Western blot analysis of human recombinant ER α (A), ER α expression in MCF7 nuclear extract (B) and T-47D whole cell lysate (C). Note amino terminal truncated form of ER α .

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 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).

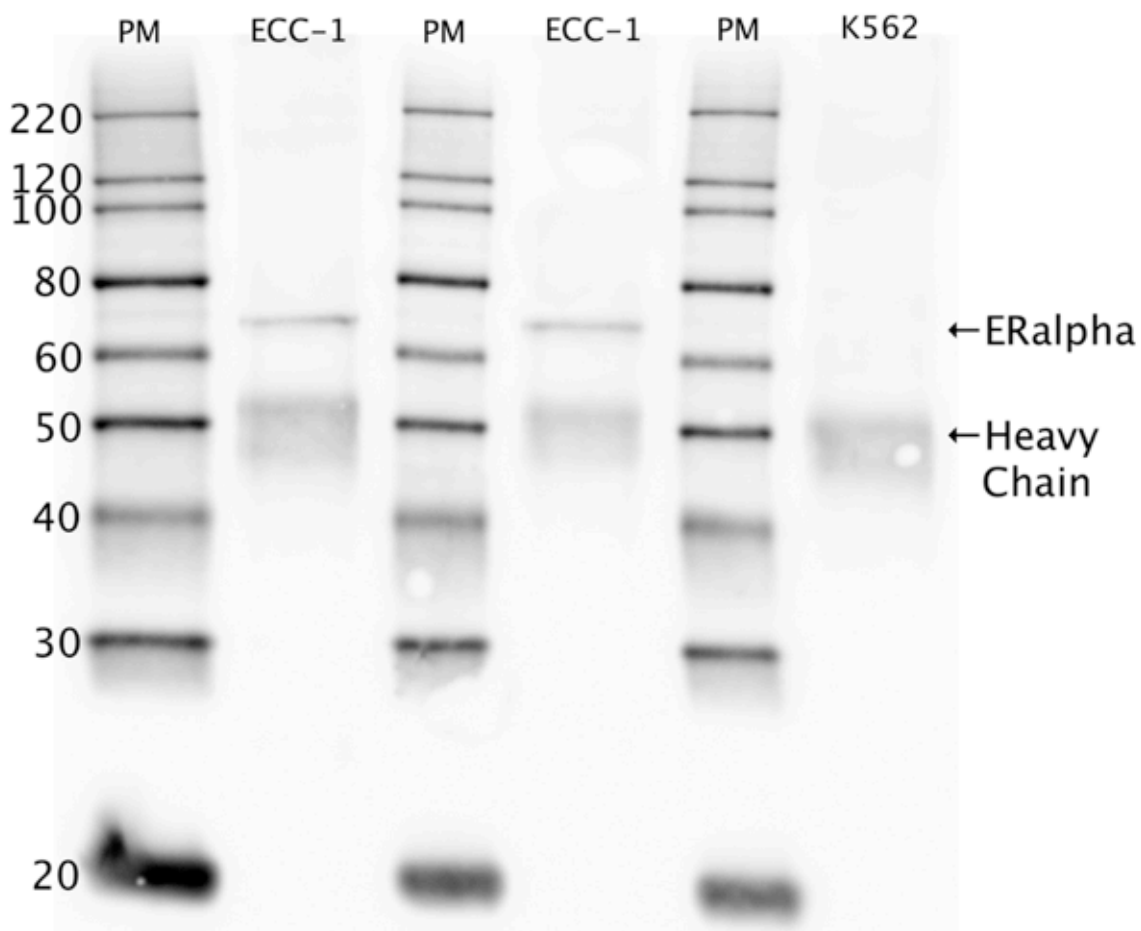


Figure Legend: ER α immunoblot: IP-western with sc-543 ER α antibody in whole cell lysates of ECC-1 and K562; ER α is not expressed in K562, and this lane is included as a negative control. Heavy chain of IgG is indicated, and ER α band is indicated at ~66 kDa.

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, ECC-1 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 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.

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

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

Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1 HSP7C_HUMAN

Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Homo sapiens GN=RPN1
PE=1 SV=1 RPN1_HUMAN

Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6 K2C1_HUMAN

T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4 TCPG_HUMAN

ATPase family AAA domain-containing protein 3A OS=Homo sapiens GN=ATAD3A PE=1 SV=2
ATD3A_HUMAN

Estrogen receptor OS=Homo sapiens GN=ESR1 PE=1 SV=2 ESR1_HUMAN

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

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

RNA-binding protein 39 OS=Homo sapiens GN=RBM39 PE=1 SV=2 RBM39_HUMAN

Guanine nucleotide-binding protein-like 3 OS=Homo sapiens GN=GNL3 PE=1 SV=2
GNL3_HUMAN

Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6 K1C10_HUMAN

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

Ribosomal L1 domain-containing protein 1 OS=Homo sapiens GN=RSL1D1 PE=1 SV=3
RSL1D1_HUMAN

Zinc finger protein 512 OS=Homo sapiens GN=ZNF512 PE=1 SV=2 ZNF512_HUMAN

Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3
HNRNPM_HUMAN

Nucleolar protein 58 OS=Homo sapiens GN=NOP58 PE=1 SV=1 NOP58_HUMAN

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

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

Lamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1 LMNA_HUMAN

26S proteasome non-ATPase regulatory subunit 3 OS=Homo sapiens GN=PSMD3 PE=1 SV=2
PSMD3_HUMAN