# ENCODE Antibody Validation Documentation
# Transcription factor: Estrogen Receptor Alpha (GeneID 2099)

**From: Myers Lab, HudsonAlpha Institute for Biotechnology**
**Contact Person:** Dr. Florencia Pauli (fpauli@hudsonalpha.org)

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

**Antibody:** ERα (HC-20), Santa Cruz Biotechnology (sc-543)
Rabbit polyclonal, epitope mapping at the C-terminus of ERα 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

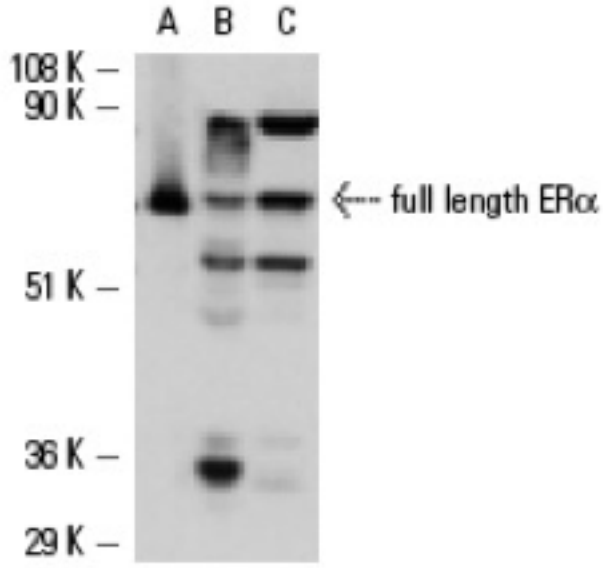


**Figure Legend:** Western blot analysis of human recombinant ERα (A), ERα expression in MCF7 nuclear extract (B) and T-47D whole cell lysate (C). Note amino terminal truncated form of ERα.

**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).

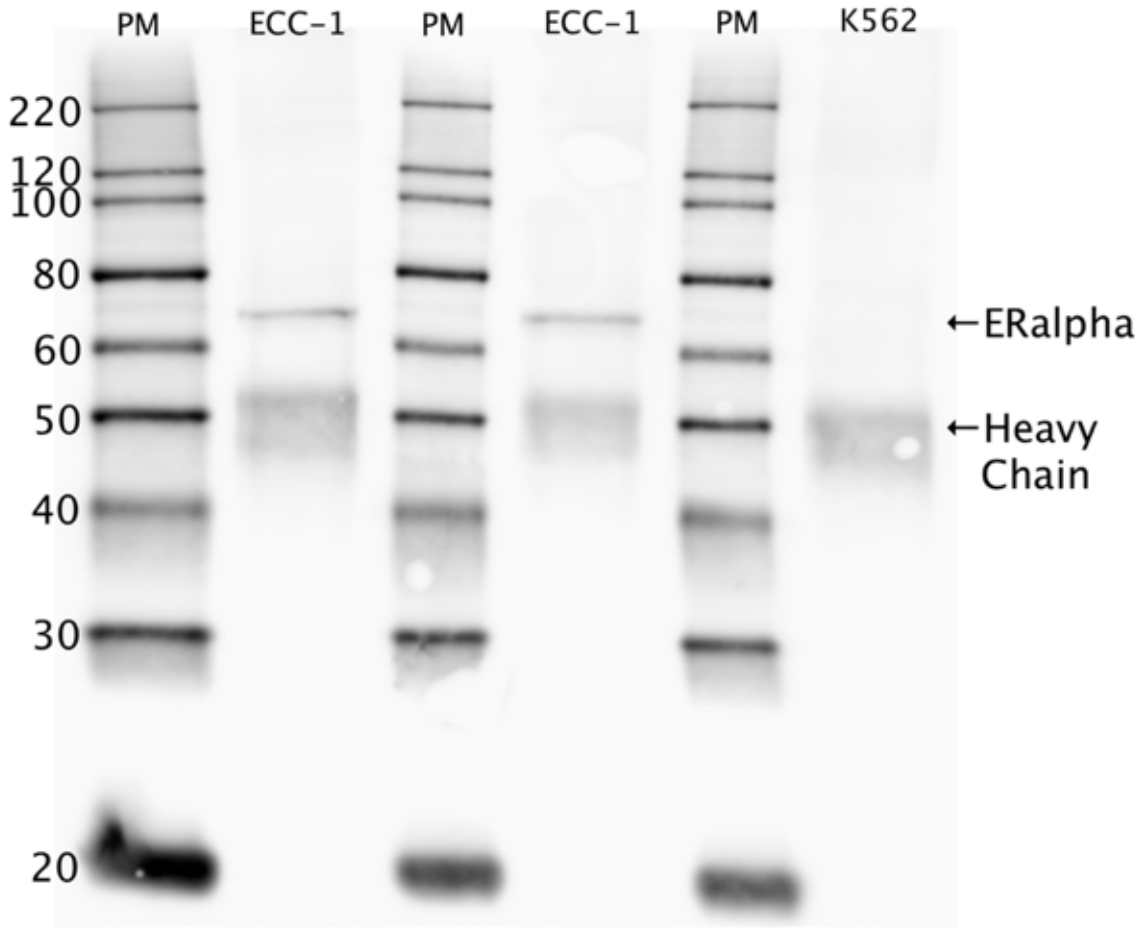


**Figure Legend:** ERα immunoblot: IP-western with sc-543 ERα antibody in whole cell lysates of ECC-1 and K562; ERα is not expressed in K562, and this lane is included as a negative control. Heavy chain of IgG is indicated, and ERα band is indicated at ~66 kDa.

## Validation 2: In progress