# ENCODE Antibody Validation Documentation
# Transcription factor: Sp1 transcription factor (GeneID 6667)

**From: Myers Lab, HudsonAlpha Institute for Biotechnology**
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**Transcription factor:** SP1 (GeneID 6667; ~80 kDa)

**Antibody:** Sp1 (E-3), Santa Cruz Biotechnology (sc-17824)
Mouse monoclonal, raised against amino acids 121-345 mapping near N-terminus of Sp1 of human origin
Web: http://www.scbt.com/datasheet-17824-sp1-e-3-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

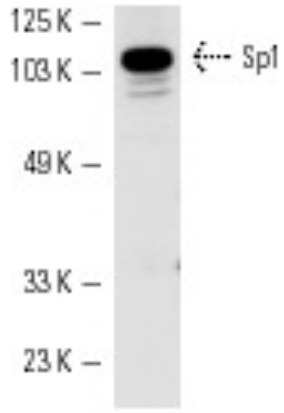


**Figure Legend:** Western blot analysis of Sp1 expression in K562 nuclear extract.

**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).

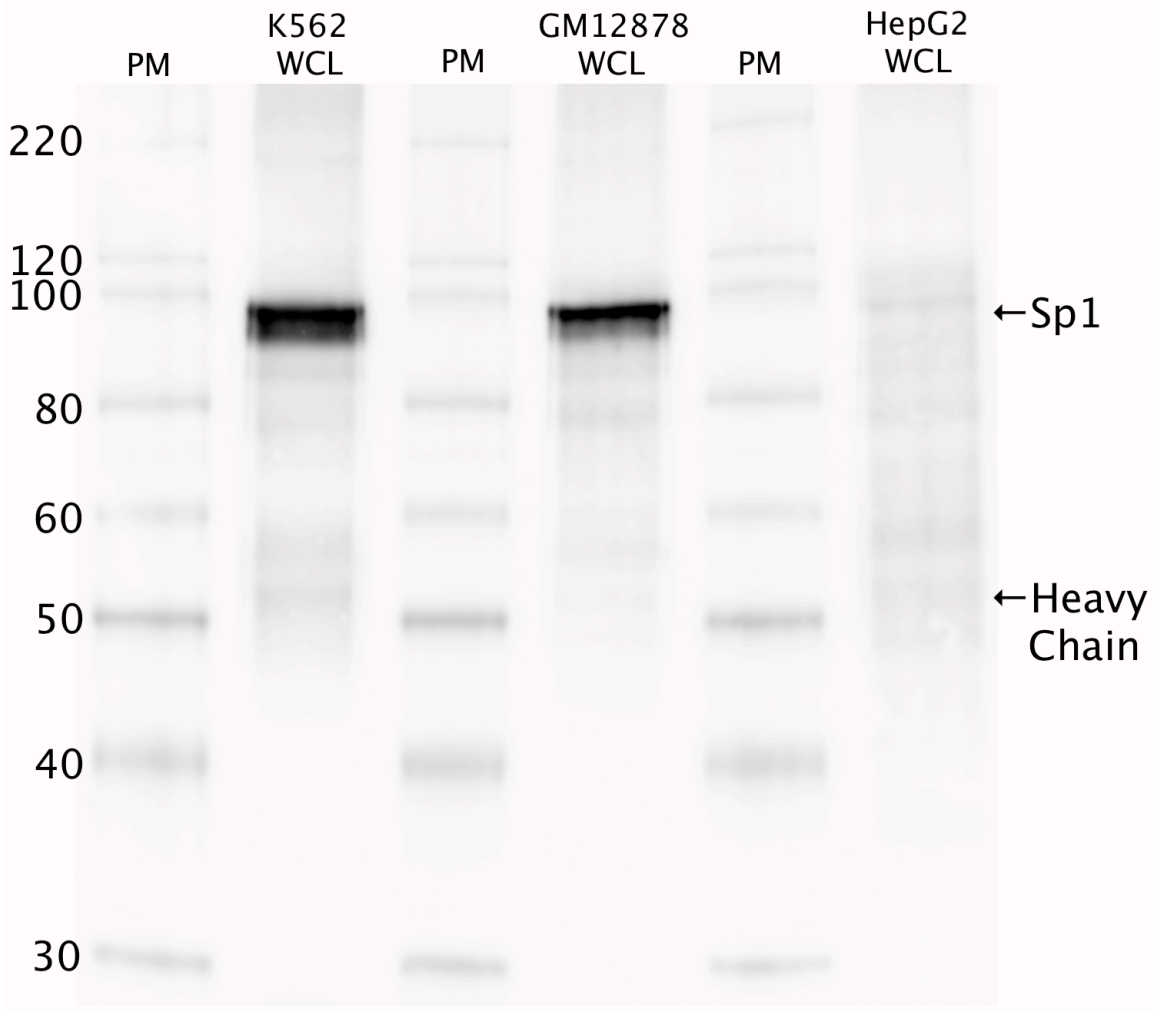


**Figure Legend:** SP1 immunoblot: IP-western with sc-17824 SP1 antibody in whole cell lysates (WCL) of K562, GM12878, and HepG2. Heavy chain of IgG is indicated, and SP1 band is indicated at ~90 kDa.

## Validation 2: In progress