

**ENCODE Antibody Validation Documentation**  
**Transcription factor: Sp4 transcription factor (GenelD 6671)**

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

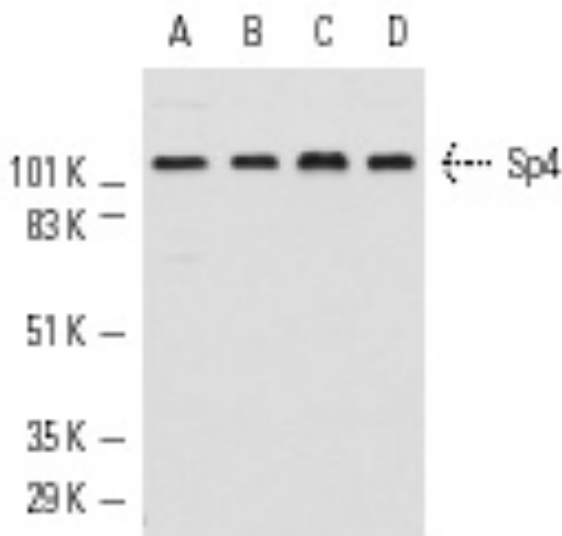
**Transcription factor: SP4 (GenelD 6671; ~82 kDa)**

**Antibody: SP4 (V-20), Santa Cruz Biotechnology (sc-645)**  
Rabbit polyclonal, epitope mapping at C-terminus of SP4 of human origin  
Web: <http://www.scbt.com/datasheet-645-sp4-v-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**

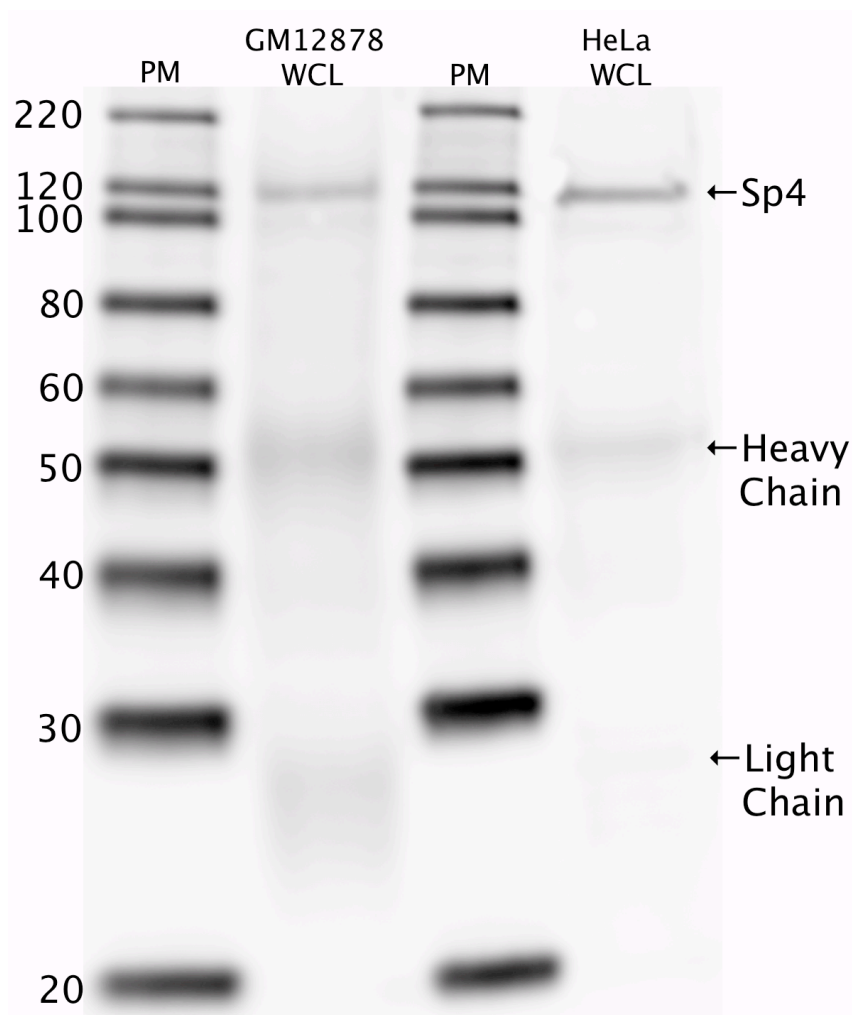


**Figure Legend:** Western blot analysis of SP4 expression in NIH/3T3 (A,B) and KNRK (C,D) nuclear extracts.

## 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:** SP4 immunoblot: IP-western with sc-645 SP4 antibody in whole cell lysates (WCL) of GM12878 and HeLa. Heavy chain and light chain of IgG are indicated, and SP4 band is indicated at ~120 kDa.

**Validation 2: In progress**