# ENCODE Antibody Validation Documentation
# Transcription factor: Spleen focus forming virus (SFFV) proviral integration oncogene spi1 (GeneID 6688)

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**Transcription factor:** SPI-1, PU.1 (GeneID 6688; ~31 kDa)

**Antibody:** PU.1 (H-135), Santa Cruz Biotechnology (sc-22805)
Rabbit polyclonal, epitope corresponding to amino acids 1-135 mapping at the N-terminus of PU.1 of human origin
Web: http://www.scbt.com/datasheet-22805-pu-1-h-135-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. 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 using a Bio-Rad Trans-Blot Electrophoretic Transfer system. Standard western blot protocol was used to probe the membrane with the primary antibody (same antibody as used for IP), and an HRP-conjugated secondary antibody and SuperSignal West Femto solution (Thermo Scientific) were used to detect the immunoprecipitated proteins.

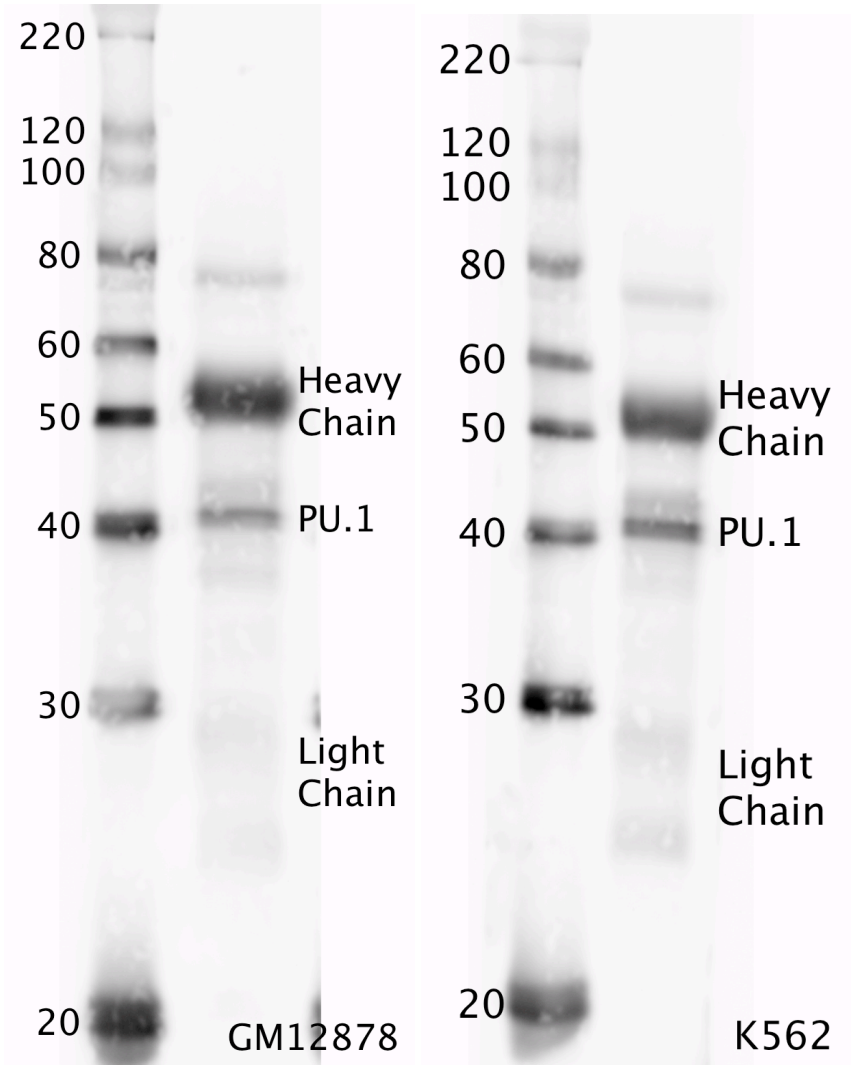


**Figure Legend:** PU.1 immunoblot: IP-western with sc-22805 PU.1 antibody in whole cell lysates of GM12878 and K562. Heavy and light chains of IgG are indicated, and PU.1 band is indicated at ~41 kDa.

## Validation 2: In progress