# ENCODE Antibody Validation Documentation
# Transcription factor: Paired box 5 (GeneID 5079)

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**Transcription factor:** PAX5 (GeneID 5079; ~42 kDa)

**Antibody:** PAX-5 (N-19), Santa Cruz Biotechnology (sc-1975)
Goat polyclonal, epitope mapping at the N-terminus of PAX-5 of human origin
Web: http://www.scbt.com/datasheet-1975-pax-5-n-19-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

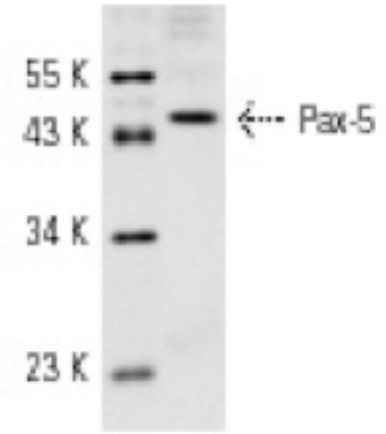



**Figure Legend:** Western blot analysis of PAX-5 expression in NAMALWA whole cell lysate.

**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 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.

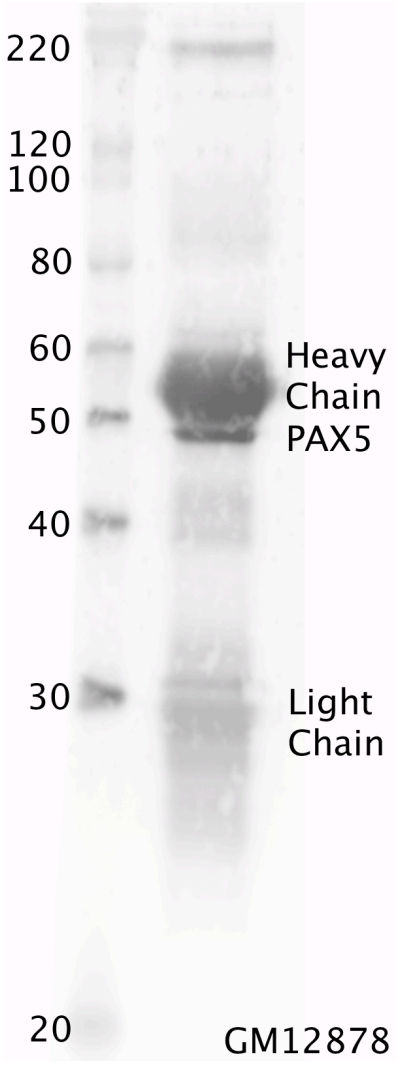


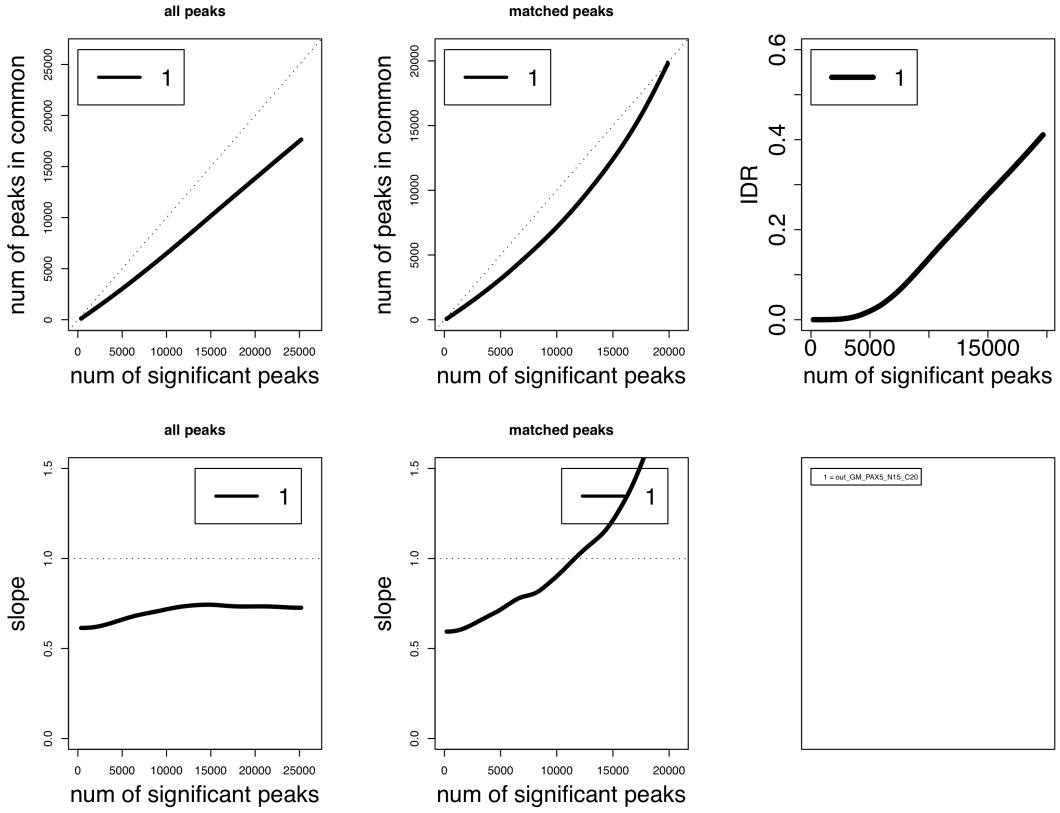

**Figure Legend:** PAX5 immunoblot: IP-western with sc-1975 PAX-5 antibody in whole cell lysate of GM12878. Heavy and light chains of IgG are indicated, and PAX5 band is indicated at ~47 kDa.

## Validation 2: Immunoprecipitation with multiple antibodies against different parts of the target protein

ENCODE data standards allow for secondary validation of antibodies by performing ChIP with multiple antibodies against different parts of the target protein. A statistically significant overlap of targets constitutes validation.

PAX5 (N-19) sc-1975 is a goat polyclonal antibody with epitope mapping at the N-terminus of PAX-5 of human origin. A second antibody used in ChIP-seq experiments on PAX5 in our lab is PAX5 (C-20) sc-1974, a goat polyclonal antibody with epitope mapping at the C-terminus of PAX-5 of human origin. Irreproducible Discovery Rate (IDR) analysis results for two ChIP-seq experiments using these two antibodies, each in GM12878, are as follows:

At IDR 0.01: 4145 peaks are significant
At IDR 0.05: 6815 peaks are significant
At IDR 0.1: 8756 peaks are significant




These results indicate significant overlap between the two ChIP-seq libraries.

## References

Li Q, Brown JB, Huang H, Bickel PJ. Measuring Reproducibility of High-throughput experiments (Submitted to the Annals of Applied Statistics)