

ENCODE Antibody Validation Documentation
Transcription factor: POU class 2 homeobox 2 (GeneID 5452)

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Transcription factor: POU2F2 (GeneID 5452; ~51 kDa)

Antibody: OCT-2 (C-20), Santa Cruz Biotechnology (sc-233)

Rabbit polyclonal, epitope mapping at the C-terminus of OCT-2 of human origin

Web: <http://www.scbt.com/datasheet-233-oct-2-c-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

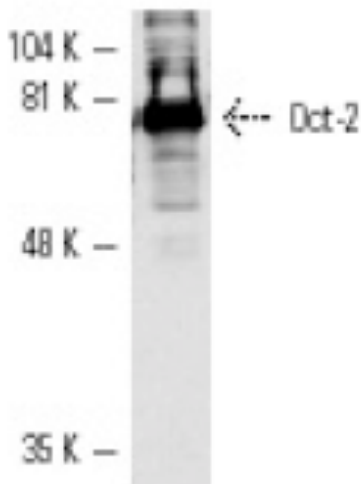


Figure Legend: Western blot analysis of OCT-2 expression in NIH/3T3 nuclear extract.

b. Myers Lab immunoblot analysis

Western blot protocol

Whole cell lysates or nuclear extracts 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).

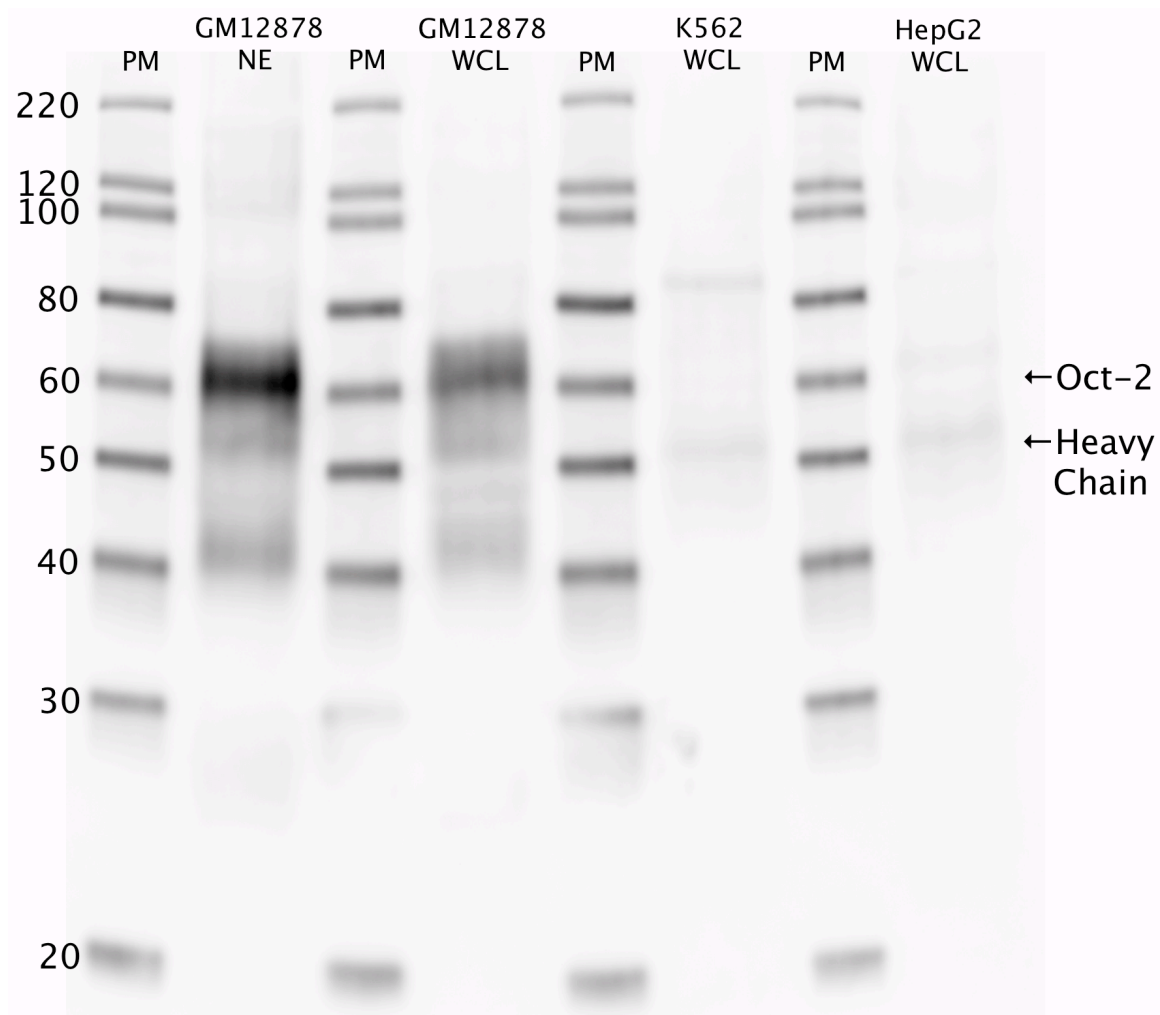


Figure Legend: POU2F2 immunoblot: IP-western with sc-233 OCT-2 antibody in nuclear extract (NE) or whole cell lysate (WCL) of, left to right, GM12878, K562, and HepG2. Heavy chain of IgG is indicated, and POU2F2 band is indicated at ~60 kDa.

Validation 2: In progress