

ENCODE Antibody Validation Documentation
**Transcription factor: nuclear receptor subfamily 3, group C,
member 1 (glucocorticoid receptor) (GeneID 2908)**

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Transcription factor: GR (GeneID 2908; ~86 kDa)

Antibody: GR (E-20), Santa Cruz Biotechnology (sc-1003)

Rabbit polyclonal, epitope mapping at the N-terminus of GR alpha of human origin

Web: <http://www.scbt.com/datasheet-1003-gr-e-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

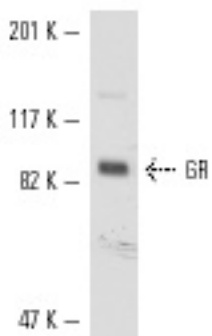


Figure Legend: Western blot analysis of GR expression in A-431 nuclear extract.

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 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.

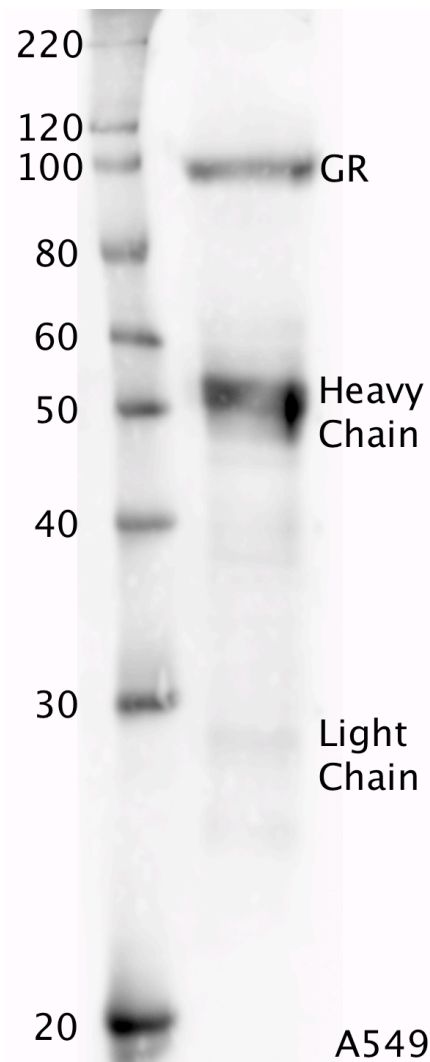


Figure Legend: GR immunoblot: IP-western with sc-1003 GR antibody in whole cell lysate of A549. Heavy chain and light chain of IgG are indicated, and GR band is indicated at ~90 kDa.

Validation 2: In progress