ENCODE Antibody Validation Documentation
Transcription factor: RAD21 homolog (S. pombe) (GeneID 5885)

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Transcription factor: RAD21 (GeneID 5885; ~72 kDa)

Antibody: RAD21, Abcam (ab992)
Lot numbers: 891751, 940739
Rabbit polyclonal, raised against synthetic peptide (human) conjugated to KLH, representing portion of human RAD21 encoded within exon 14
Web: http://www.abcam.com/Rad21-antibody-ChIP-Grade-ab992.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

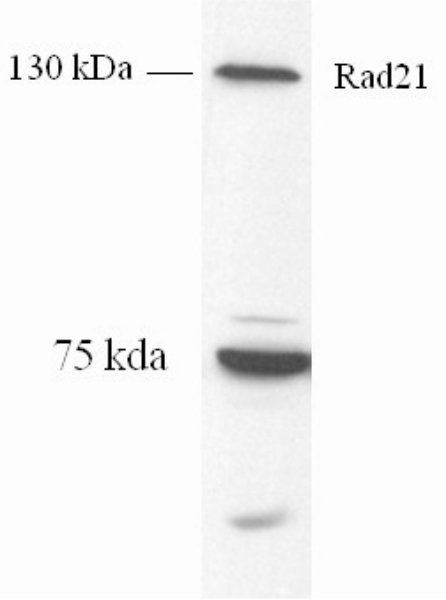

Figure Legend: Western blot analysis of RAD21 expression in HeLa whole cell extract.

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 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).

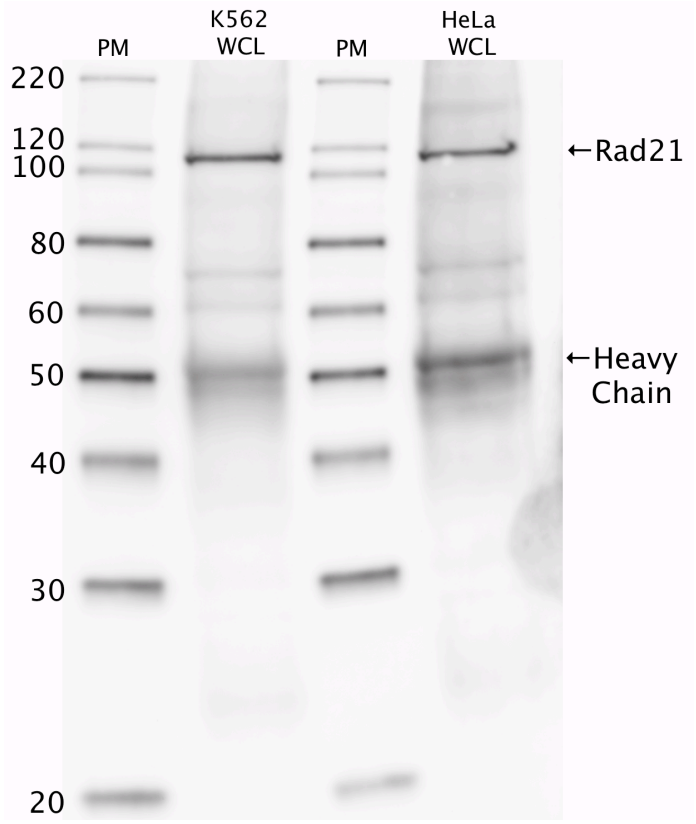

Figure Legend: RAD21 immunoblot: IP-western with ab992 RAD21 antibody in whole cell lysates (WCL) of K562 and HeLa. Heavy chain of IgG is indicated, and RAD21 band is indicated at ~120 kDa.

Validation 2: In progress