# ENCODE DCC Antibody Validation Document 

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Lab
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Antibody Name: BRCA1 (A300-000A) Target: BRCA1 (Entrez GeneID 672)

Company/
Source:
Bethyl Laboratories

Catalog Number, database ID, laboratory A300-000A Lot Number A300-000A-2
Antibody $\quad \begin{aligned} & \text { Rabbit polyclonal recognizing a region between residue } 1800 \text { and the C-terminus (residue 1863) of } \\ & \text { human Breast Cancer Gene 1. Antibody Target: BRCA1 }\end{aligned}$ Description: human Breast Cancer Gene 1. Antibody Target: BRCA1

Target
Nuclear phosphoprotein that plays a role in maintaining genomic stability, and acts as a tumor Description. $\left\lvert\, \begin{gathered}\text { suppressor. Associates with RNA polymerase II, and through the C-terminal domain, also interacts }\end{gathered}\right.$ Description: with histone deacetylase complexes. Plays a role in transcription, DNA repair of double-stranded breaks, and recombination. Mutations in this gene are responsible for approximately $40 \%$ of inherited breast cancers and more than $80 \%$ of inherited breast and ovarian cancers.
$\square$


Reference (PI/ Publication
Information)


Please complete the following for antibodies to histone modifications:
if your specifications are not listed in the drop-down box,
please write-in the appropriate information
$\square$
$\square$ AA Position $\square$
Modification $\square$

Western blots on nuclear lysates from cell lines GM12878 (Lane1), K562 (Lane2), HeLaS3 (Lane3), and HepG2 (Lane4).
A band of $\sim 207 \mathrm{kD}$ is detected by Western blotting with A300-000A in K562 and HelaS3 nuclear lysates.

Validation \#1
Analysis
$\square$


Western Blot analysis of nuclear extracts from GM12878, K562, HelaS3 and HepG2 using anti BRCA1 antibody from Bethyl Labs (A300-000A). Expected protein band 207.7 KDa

Immunoprecipitation of BRCA1 from K562 cells using A300-000A. Lane 1: input nuclear lysate, Lane 2: material immunoprecipitated with A300-000A, Lane 3: material immunoprecipitated using control $\operatorname{lgG}$. Bands A was excised from the gel and subject to analysis by mass spectrometry.
IP followed by masspectrometry: Briefly, protein was immunoprecipitated from K562 whole cell lysates using A300-000A, and the IP fraction was loaded on a $10 \%$ polyacrylamide gel (NuPAGE Bis-Tris Gel) and separated with an Invitrogen NuPAGE electrophoresis system. The gel was silver-stained, gel fragments corresponding to the bands indicated were excised and destained using the SilverSNAP Stain for Mass Spectrometry (Pierce). Then proteins were trypsinized using the in-gel digestion method. Digested proteins were analyzed on an LTQ-Orbitrap (Thermo Scientific) by the nanoLC-ESI-MS/MS technique. Peptides were identified by the SEQUEST algorithm and filtered with a high confidence threshold (Protein false discovery rate $<1 \%, 2$ peptides per protein minimum).
We report 11 proteins identified in band A, although 3 of these are also present in a control immunoprecipitation and are thus likely to present due to non-specific association with the IP matrix. Of the specifically immunoprecipitated proteins, BRCA1 and its isoforms 1, 2 and 4 are the most abundant ( 25 peptides).
Based on these observations, this band is likely due to the presence of immunoprecipitated BRCA1 and A300-000A meets the ENCODE standard for validation by this criterion.

Immunoprecipitation assay (IP) + mass spectrometry assay

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\text { MW } \quad \text { BRCA1 (A300-000A) (R) } 207.7 \text { kD }
$$



| Spectrum | Name of Protein | Count of Peptides | Ratio(BRCA1/IgG Control) |
| :--- | :--- | :---: | :--- |
| BRCA1_Band A | Isoform 1 of DNA-dependent protein kinase catalytic subunit | 8 | NOT IN CONTROL IP |
| BRCA1_Band A | Isoform 2 of DNA-dependent protein kinase catalytic subunit | 8 | NOT IN CONTROL IP |
| BRCA1_Band A | BRCA1 protein | 7 | NOT IN CONTROL IP |
| BRCA1_Band A | similar to protein kinase, DNA-activated, catalytic polypeptide | 7 | NOT IN CONTROL IP |
| BRCA1_Band A | IRIS | 6 | NOT IN CONTROL IP |
| BRCA1_Band A | Isoform 1 of Breast cancer type 1 susceptibility protein | 6 | NOT IN CONTROL IP |
| BRCA1_Band A | Isoform 2 of Breast cancer type 1 susceptibility protein | 6 | NOT IN CONTROL IP |
| BRCA1_Band A | Isoform 4 of Breast cancer type 1 susceptibility protein | 6 | NOT IN CONTROL IP |
| BRCA1_Band A | Isoform 1 of Cell division cycle and apoptosis regulator protein 1 | 5 | NOT IN CONTROL IP |
| BRCA1_Band A | Isoform 2 of Cell division cycle and apoptosis regulator protein 1 | 5 | NOT IN CONTROL IP |
| BRCA1_Band A | CDNA FL554020, highly similar to Heterogeneous nuclear ribonucleoprotein U | 4 | 0.666666667 |
| BRCA1_Band A | HNRPU protein | 4 | 0.666666667 |
| BRCA1_Band A | Isoform 1 of Nucleolar RNA helicase 2 | 4 | NOT IN CONTROL IP |
| BRCA1_Band A | Isoform 2 of Nucleolar RNA helicase 2 | 4 | NOT IN CONTROL IP |
| BRCA1_Band A | Isoform Long of Heterogeneous nuclear ribonucleoprotein U | 4 | 0.6666666667 |
| BRCA1_Band A | Isoform Short of Heterogeneous nuclear ribonucleoprotein U | 4 | 0.666666667 |
| BRCA1_Band A | Nuclear pore complex protein Nup205 | 3 | NOT IN CONTROL IP |

