

RbBP5 Antibody

Rabbit Polyclonal

Antigen Affinity Purified

Protein ID NP_005048.2

Catalog No. A300-109A

GeneID 5929

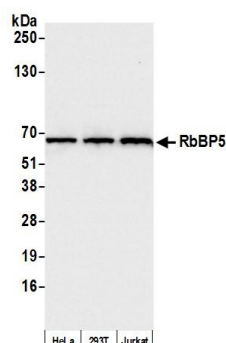
Lot No. A300-109A-3



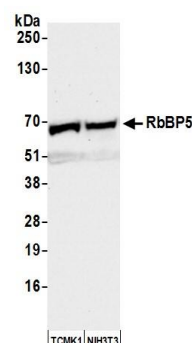
APPLICATIONS	WB, IP, IHC, ICC, ChIP, ChIP-chip, ChIP-Seq														
SPECIES REACTIVITY	Human, Mouse														
AMOUNT	100 µl														
CONCENTRATION	1000 µg/ml														
STORAGE/SHELF LIFE	2 – 8° C / 1 year from date of receipt														
PHYSICAL STATE	Liquid														
BUFFER	Tris-citrate/phosphate buffer, pH 7 to 8 containing 0.09% Sodium Azide														
ISOTYPE	IgG														
ORIGIN	USA														
PRODUCTION PROCEDURES	<p>Antibody was affinity purified using an epitope specific to RbBP5 immobilized on solid support.</p> <p>The epitope recognized by A300-109A maps to a region between residue 500 and the C-terminus (residue 538) of human retinoblastoma binding protein 5 using the numbering given in entry NP_005048.2 (GeneID 5929).</p> <p>Immunoglobulin concentration was determined by extinction coefficient: absorbance at 280 nm of 1.4 equals 1.0 mg of IgG.</p>														
APPLICATIONS	<p>Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use.</p> <table><tr><td>Western Blot</td><td>1:10,000 – 1:25,000</td></tr><tr><td>Immunoprecipitation</td><td>2 – 10 µg/mg lysate</td></tr><tr><td>Immunohistochemistry</td><td>1:500 to 1:2,000. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections.</td></tr><tr><td>Immunocytochemistry</td><td>1:250 – 1:1,000</td></tr><tr><td>ChIP</td><td>1 – 3 µg as per Dou et al., Nat Struct Mol Biol 13 (8):713-719, 2006. Previous lots of this antibody have performed in this application.</td></tr><tr><td>ChIP-chip</td><td>10 µg. Previous lots of this antibody have performed in this application.</td></tr><tr><td>ChIP-Seq</td><td>2 µg. Previous lots of this antibody have performed in this application.</td></tr></table>	Western Blot	1:10,000 – 1:25,000	Immunoprecipitation	2 – 10 µg/mg lysate	Immunohistochemistry	1:500 to 1:2,000. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections.	Immunocytochemistry	1:250 – 1:1,000	ChIP	1 – 3 µg as per Dou et al., Nat Struct Mol Biol 13 (8):713-719, 2006. Previous lots of this antibody have performed in this application.	ChIP-chip	10 µg. Previous lots of this antibody have performed in this application.	ChIP-Seq	2 µg. Previous lots of this antibody have performed in this application.
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APPLICATION NOTES	Western blot of lysates performed using standard western blot reagents and 4-20% SDS-PAGE.														
IHC HUMAN CONTROLS	Colon Carcinoma														
ADDITIONAL INFO	<p>https://www.bethyl.com/product/A300-109A</p> <p>Use the link above to view SDS, a current list of citations, and other product specific information.</p>														

This document certifies that this product has met all of the quality control standards defined by Bethyl Laboratories, Inc.
Eric McIntush, PhD | Chief Scientific Officer

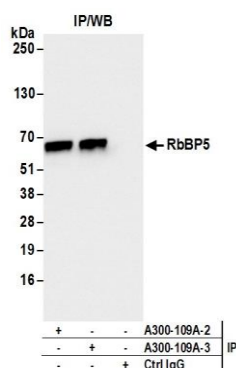
Date: July 31, 2019



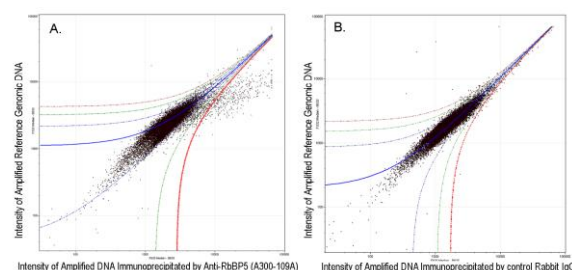
Detection of human RbBP5 by western blot. *Samples:* Whole cell lysate (15 µg) from HeLa, HEK293T, and Jurkat cells prepared using NETN lysis buffer. *Antibody:* Affinity purified rabbit anti-RbBP5 antibody A300-109A (lot A300-109A-3) used for WB at 0.04 µg/ml. *Detection:* Chemiluminescence with an exposure time of 3 seconds.



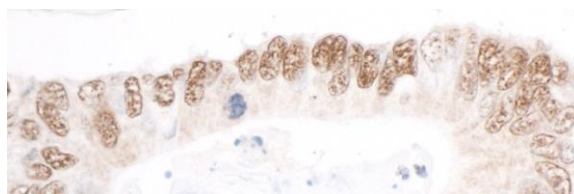
Detection of mouse RbBP5 by western blot. *Samples:* Whole cell lysate (50 µg) from TCMK-1 and NIH 3T3 cells prepared using NETN lysis buffer. *Antibody:* Affinity purified rabbit anti-RbBP5 antibody A300-109A (lot A300-109A-3) used for WB at 0.04 µg/ml. *Detection:* Chemiluminescence with an exposure time of 10 seconds.



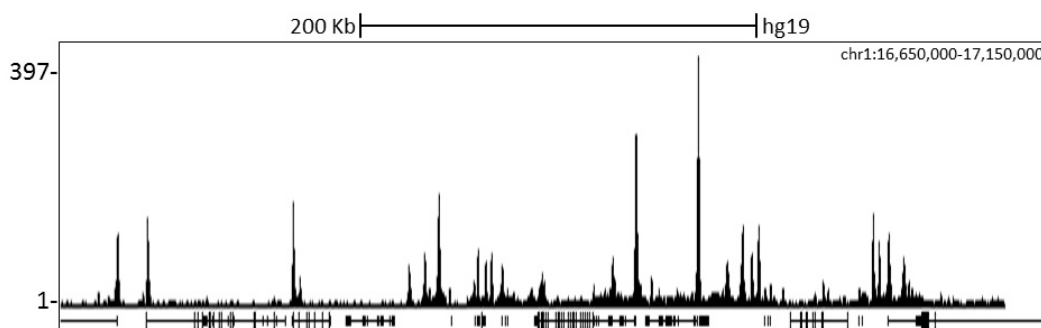
Detection of human RbBP5 by western blot of immunoprecipitates. *Samples:* Whole cell lysate (1.0 mg per IP reaction; 20% of IP loaded) from HeLa cells prepared using NETN lysis buffer. *Antibodies:* Affinity purified rabbit anti-RbBP5 antibody A300-109A (lot A300-109A-3) used for IP at 6 µg per reaction. RbBP5 was also immunoprecipitated by a previous lot of this antibody (lot A300-109A-2). For blotting immunoprecipitated RbBP5, A300-109A was used at 0.04 µg/ml. *Detection:* Chemiluminescence with an exposure time of 1 second.



ChIP-chip scatter plot of anti-RbBP5 (A300-109A) enriched DNA binding sites versus input reference DNA. A. 10 µg of A300-109A was used to immunoprecipitate chromatin from K-562 cells according to Ren et al (Genes Dev. 2002 16: 245-256). immunoprecipitated DNA and reference DNA were amplified via ligation-mediated PCR and the products labeled with fluorescent dNTPs. The labeled ChIP and reference DNA were pooled, hybridized to a DNA microarray, and analyzed. Data points below the +3 SD curve (red line) represent significantly enriched binding sites. B. As a control, a similar experiment was performed using normal rabbit IgG. Compared to the anti-RbBP5 ChIP, normal rabbit IgG showed little enrichment.

**Detection of human RbBP5 by immunohistochemistry.**

Sample: FFPE section of human colon carcinoma. *Antibody:* Affinity purified rabbit anti- RbBP5 (Cat. No. A300-109A lot 3) used at a dilution of 1:1,000 (1 µg/ml). *Detection:* DAB



Localization of RbBP5 Binding Sites by ChIP-seq. Chromatin from K562 cells was immunoprecipitated with anti-RbBP5 antibody A300-109A and analyzed by DNA sequencing. The figure illustrates the peak distribution of RbBP5 binding within a 500 Kb region of chromosome 1 as detected using anti-RbBP5 antibody A300-109A. ChIP-seq validation performed by Diogenode, Denville, NJ.