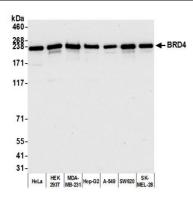
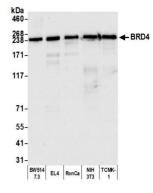
## **BRD4** Antibody

Rabbit Polyclonal Antigen Affinity Purified Catalog No. A301–985A50 Lot No. A301–985A50–8		Protein ID GenelD	NP_490597.1 23476		BETHYL LABORATORIES, INC	
APPLICATIONS		WB, IP, IHC				
SPECIES REACTIVITY		Human, Mouse				
AMOUNT		50 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		Antibody was affinity purified using an epitope specific to BRD4 immobilized on solid support.				
PROCEDURES		The epitope recognized by A301-985A50 maps to a region between residue 1312 and 1362 of human bromodomain-containing protein 4 using the numbering given in entry NP_490597.1 (GeneID 23476). Immunoglobulin concentration was determined by extinction coefficient: absorbance at 280 nm of 1.4 equals 1.0 mg of IgG.				
		Centrifuge tube to remove product from lid. Optimal working dilutions should be determined experimentally by the investigator. Prepare working dilution immediately before use.				
		Western Blot	1:2	2,000 - 1:10,000		
		Immunoprecipit	tation 2 -	5 µg/mg lysate		
		Immunohistoch		,000 – 1:5,000. Epitope rei commended for FFPE tissue		buffer pH 6.0 is
APPLICATION NOTES		Western blot of immunoprecipitates performed using Normal Pig Serum (Cat. No. S100–020), Goat anti–Rabbit Light Chain HRP Conjugate (Cat. No. A120–113P) and 4–8% SDS–PAGE (link to IP–western blot protocol in Additional Info section below).				
			of lysates performed using standard western blot reagents and 4–8% SDS–PAGE. ma, Ovarian Carcinoma			
IHC MOUSE CONTROLS		Renal Cell Carcinoma				
ADDITIONAL INFO		https://www.bethyl.com/product/A301-985A50 Use the link above to view SDS, a current list of citations, and other product specific information. IP-western blot protocol https://www.bethyl.com/content/protocol_IP_WB				

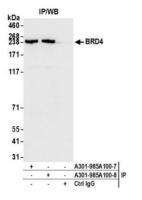
This document certifies that this product has met all of the quality control standards defined by Bethyl Laboratories, Inc. Eric McIntush, PhD | Chief Scientific Officer Date: December 20, 2019



**Detection of human BRD4 by western blot.** *Samples:* Whole cell lysate (10 µg) from HeLa, HEK293T, MDA-MB-231, Hep-G2, A-549, SW620, and SK-MEL-28 cells prepared using NETN lysis buffer. *Antibody:* Affinity purified rabbit anti-BRD4 antibody A301-985A50 (lot A301-985A50-8) used for WB at 0.04 µg/ml. *Detection:* Chemiluminescence with an exposure time of 10 seconds.

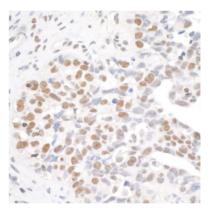


Detection of mouse BRD4 by western blot. Samples: Whole cell lysate (15  $\mu$ g) from BW5147.3, EL4, RenCa, NIH 3T3, and TCMK-1 cells prepared using NETN lysis buffer. Antibody: Affinity purified rabbit anti-BRD4 antibody A301-985A50 (lot A301-985A50-8) used for WB at 0.04  $\mu$ g/ml. Detection: Chemiluminescence with an exposure time of 3 seconds.



## Detection of human BRD4 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-BRD4 antibody A301–985A50 (lot A301–985A50–8) used for IP at 6 µg per reaction. BRD4 was also immunoprecipitated by a previous lot of this antibody (lot A301–985A50–7). For blotting immunoprecipitated BRD4, rabbit anti-BRD4 recombinant monoclonal antibody A700–004 was used at 1:1000. *Detection:* Chemiluminescence with an exposure time of 1 second.

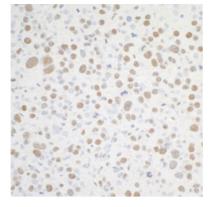


**Detection of human BRD4 by immunohistochemistry.** *Sample:* FFPE section of human ovarian carcinoma. *Antibody:* Affinity purified rabbit anti-BRD4 (Cat. No. A301-985A50 lot 8) used at a dilution of 1:5,000 (0.2 µg/ml). *Detection:* DAB



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**Detection of mouse BRD4 by immunohistochemistry.** *Sample:* FFPE section of mouse renal cell carcinoma. *Antibody:* Affinity purified rabbit anti-BRD4 (Cat. No. A301-985A50 lot 8) used at a dilution of 1:5,000 (0.2 µg/ml). *Detection:* DAB

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