BRD4 Recombinant Monoclonal Antibody [BL-149-2H5]

Rabbit Recombinant Monoclonal

Purified		Protein ID	NP_490597.1
Catalog No.	A700-004	GeneID	23476
Lot No.	A700-004-4		

APPLICATIONS	WB, IP, IHC, ICC, ICC-IF, ChIP-Seq, F
SPECIES REACTIVITY	Human, Mouse
AMOUNT	100 µl (10 blots)
CONCENTRATION	100 µg/ml
STORAGE/SHELF LIFE	2 - 8°C / 1 year from date of receipt
PHYSICAL STATE	Liquid
BUFFER	Borate Buffered Saline (BBS) pH 8.2 with 0.1% BSA and 0.09% Sodium Azide
ISOTYPE	IgG
CLONE #	BL-149-2H5
ORIGIN	USA
PRODUCTION PROCEDURES	Recombinant antibody was purified from cell culture supernatant. The epitope recognized by A700-004 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).
APPLICATIONS	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	1:1000
Immunoprecipitation	20 µl/mg lysate
Immunohistochemistry	1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE tissue sections.
Immunocytochemistry	1:100 to 1:500. Epitope retrieval with citrate buffer pH6.0 is recommended for FFPE cell sections.
Immunofluorescence (ICC)	1:25 - 1:250. Formaldehyde fixation is recommended. Permeabilization with Triton-X 100 is recommended for formaldehyde-fixed cells.
ChIP-Seq	A previous lot has qualified for ChIP-Seq. 10 - 40 µl per 30 µg chromatin.
Flow Cytometry	Fixed in 4% formaldehyde and permeabilized with 90% methanol. 1 µl per 1 x 10^6 cells.

IHC HUMAN CONTROLS	Bladder Cell Carcinoma, Breast Carcinoma, Ovarian Carcinoma, Prostate Carcinoma, Tonsil, A2780 Cells, A-549 Cells, HEK293T Cells, HeLa Cells, Jurkat Cells, KG-1 Cells, MJ Cells, OVCAR-8 Cells
ADDITIONAL INFO	https://www.bethyl.com/product/A700-004 Use the link above to view SDS, a current list of citations, and other product specific information.

This document certifies that this product has met all of the quality control standards defined by Bethyl Laboratories, Inc.

Michael Spencer, PhD — Date: October 27, 2021

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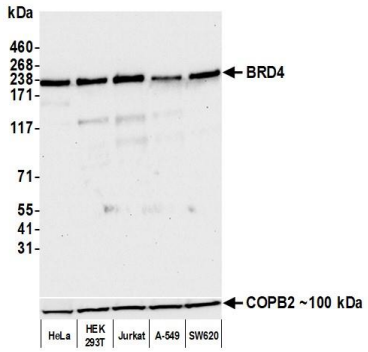
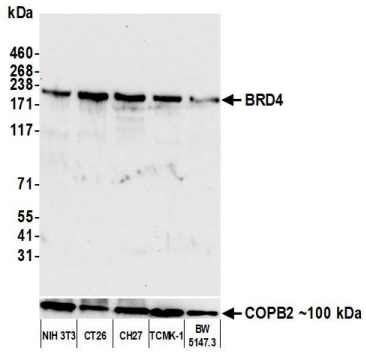
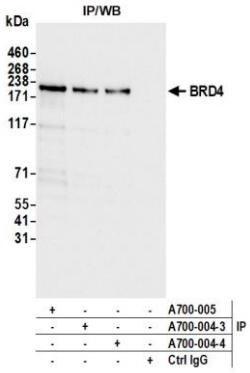


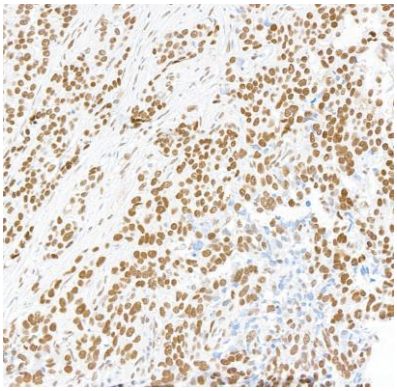
Detection of human BRD4 by western blot. *Samples:* Whole cell lysate (25 µg) from HeLa, HEK293T, Jurkat, A-549, and SW620 cells prepared using NETN lysis buffer. *Antibody:* Rabbit anti-BRD4 recombinant monoclonal antibody [BL-149-2H5] (A700-004 lot 4) used at 1:1000. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). Chemiluminescence with an exposure time of 10 seconds. Lower Panel: Rabbit anti-COPB2 antibody (A304-523A).



Detection of mouse BRD4 by western blot. *Samples:* Whole cell lysate (10 µg) from NIH 3T3, CT26, CH27, TCMK-1, and BW5147.3 cells prepared using NETN lysis buffer. *Antibody:* Rabbit anti-BRD4 recombinant monoclonal antibody [BL-149-2H5] (A700-004 lot 4) used at 1:1000. *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-101P). Chemiluminescence with an exposure time of 10 seconds. Lower Panel: Rabbit anti-COPB2 antibody (A304-523A).



Detection of human BRD4 by western blot of immunoprecipitates. *Samples:* Whole cell lysate (1.0 mg per IP reaction; 5% of IP loaded) from HeLa cells prepared using NETN lysis buffer. *Antibodies:* Rabbit anti-BRD4 recombinant monoclonal antibody [BL-149-2H5] (A700-004 lot 4) used for IP at 20 µl/mg lysate. BRD4 was also immunoprecipitated by a previous lot of this antibody (A700-004 lot 3) and a second antibody against a different epitope of BRD4 (A700-005). For blotting immunoprecipitated BRD4, A700-004 was used at 1:1000. Chemiluminescence with an exposure time of 3 seconds.



Detection of human BRD4 by immunohistochemistry. *Sample:* FFPE section of breast carcinoma. *Antibody:* Rabbit anti-BRD4 recombinant monoclonal antibody (A700-004-4). *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P).

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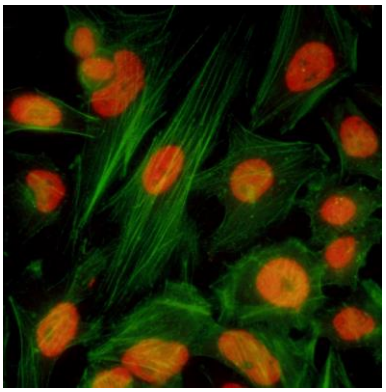
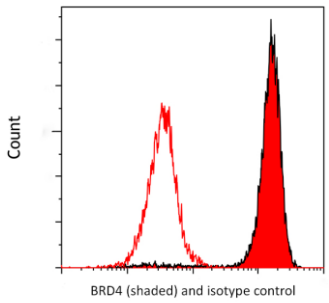
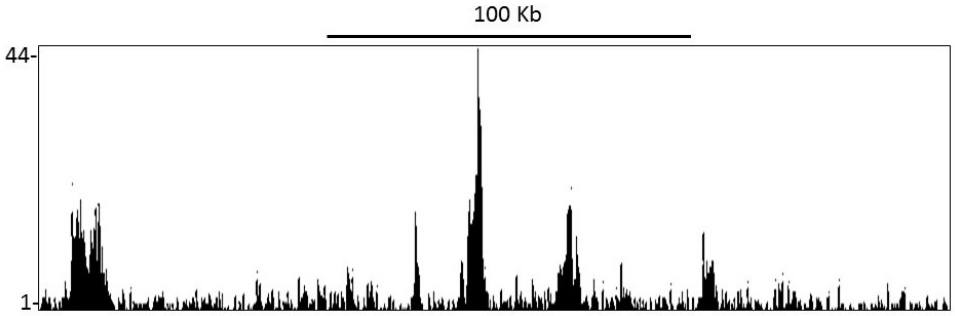
Detection of human BRD4 by immunocytochemistry. *Sample:* FFPE section of HeLa cells. *Antibody:* Rabbit anti-BRD4 recombinant monoclonal antibody (A700-004-4). *Secondary:* HRP-conjugated goat anti-rabbit IgG (A120-501P).



Detection of human BRD4 by immunocytochemistry. *Sample:* Formaldehyde-fixed HeLa cells. *Antibody:* Rabbit anti-BRD4 recombinant monoclonal antibody [BL-149-2H5] (A700-004 lot 2) used at 1:250. *Secondary:* DyLight® 550-conjugated goat anti-rabbit IgG cross-adsorbed antibody (A120-201D3).



Detection of human BRD4 (shaded) in KG-1 cells by flow cytometry. *Antibody:* Rabbit anti-BRD4 recombinant monoclonal [BL-149-2H5] (A700-004 lot 3) or isotype control (unshaded). *Secondary:* DyLight® 650-conjugated goat anti-rabbit IgG (A120-201D5).



Localization of BRD4 Binding Sites by ChIP-sequencing. Chromatin from Mia PaCa-2 cells was immunoprecipitated with anti-BRD4 antibody A700-004 and analyzed by DNA sequencing. The figure illustrates the peak distribution of BRD4 binding within a 250 Kb region of chromosome 7 as detected using anti-BRD4 antibody A700-004. ChIP-seq validation performed by Active Motif, Carlsbad, CA.

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