

BRD4 (E2A7X) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, IHC-P, ChIP, ChIP-seq, C&R, C&T, eCLIP	H	Endogenous	200	Rabbit IgG	#O60885	23476

Product Usage Information

For optimal ChIP and ChIP-seq results, use 10 µl of antibody and 10 µg of chromatin (approximately 4 x 10⁶ cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

The CUT&RUN dilution was determined using CUT&RUN Assay Kit #86652.

The CUT&Tag dilution was determined using CUT&Tag Assay Kit #77552.

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:20000 - 1:80000
Chromatin IP	1:50
Chromatin IP-seq	1:50
CUT&RUN	1:50
CUT&Tag	1:50
eCLIP	1:200

For more information about the RBP-eCLIP service please visit Eclipsebio.

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #35632.

Specificity/Sensitivity

BRD4 (E2A7X) Rabbit mAb recognizes endogenous levels of total BRD4 protein. This antibody specifically recognizes the BRD4 long isoform (UniProt #O60885-1) and does not recognize other BRD4 isoforms.

Species predicted to react based on 100% sequence homology

Bovine, Dog, Pig

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu1129 of human BRD4 protein.

Background

Bromodomain-containing protein 4 (BRD4) is a member of the bromodomains and extra terminal (BET) family of proteins, which also includes BRD2, BRD3, and BRDT (1-3). BET family proteins contain two tandem bromodomains and an extra terminal (ET) domain, and bind acetyl lysine residues (3). BRD4 is a chromatin-binding protein with a preference for Lys14 on histone H3 as well as Lys5 and Lys12 on histone H4 (4). BRD4 chromatin binding occurs throughout the cell cycle, including condensed mitotic chromosomes, when the majority of genes are silenced (5). BRD4 association with chromatin during mitosis is thought to be an important part of the bookmarking mechanism to accelerate reactivation of the silenced genes upon exit from mitosis (2,6). BRD4 has been shown to facilitate transcription by recruiting the positive transcription elongation factor b (pTEFb) complex that phosphorylates Ser2 of the heptapeptide repeat of the carboxy-terminal domain of RNA polymerase II, promoting transcription elongation (3,7,8). In addition, BRD4 has been found to be part of the super elongation complex and the polymerase associated factor complex (PAFc) in MLL-fusion derived leukemia cell lines, demonstrating a role for BRD4 in the regulation of transcription elongation (9). Research studies have shown that BRD4 (and BET family proteins) may be promising therapeutic targets for various Myc-driven cancers, such as Burkitt's lymphoma and certain acute myeloid leukemias (1,10,11). Investigators have found molecular inhibition of BET proteins to be effective in inducing apoptosis in various MLL-fusion driven leukemic cell lines by competing BRD3 and BRD4 from chromatin, leading to reduced expression of Bcl-2, Myc, and CDK6 (9). BET inhibition has also been shown to have antitumor

activities against nuclear protein in testis (NUT) midline carcinoma cell lines and xenografts in mice where BRD4 is found to be a frequent translocation partner of the NUT protein (12). In addition, BRD4 regulates the expression of some inflammatory genes, and inhibition of BRD4 (and BET family proteins) chromatin binding causes reduced expression of a subset of inflammatory genes in macrophages, leading to protection against endotoxic shock and sepsis (13).

Background References

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5. Dey, A. et al. (2009) *Mol Biol Cell* 20, 4899-909.
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8. Yang, Z. et al. (2005) *Mol Cell* 19, 535-45.
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12. Filippakopoulos, P. et al. (2010) *Nature* 468, 1067-73.
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Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin) **ChIP:** Chromatin IP **ChIP-seq:** Chromatin IP-seq **C&R:** CUT&RUN **C&T:** CUT&Tag **eCLIP:** eCLIP

Cross-Reactivity Key

H: Human

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