Store at -20°C

#11974

RNF20 (D6E10) XP® Rabbit mAb

www.cellsignal.com

Small 100 µl (10 western blots)
Petite 40 µl (4 western blots)

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rev. 06/17/14

Entrez-Gene ID #56254
UniProt ID #Q5VTR2

For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, ChIP Endogenous	H, M, R, Mk, (Hm, Dg, Pg, Hr)	120 kDa	Rabbit IgG**

Background: In mammalian cells, the significance of histone H2B ubiquitination in chromatin epigenetics came from the identification of the budding yeast protein Bre1 (1,2). Together with the ubiquitin-conjugating enzyme Rad6, Bre1 serves as the E3 ligase in the monoubiquitination of the yeast histone H2B within transcribed regions of chromatin (1-3). Subsequently, the mammalian orthologs of yeast Bre1, RNF20 and RNF40, were identified (4,5). These two proteins form a tight heterodimer that acts as the major E3 ligase responsible for histone H2B monoubiquitination at Lys120 in mammalian cells, a modification linked to RNA Pol II-dependent transcription elongation in undamaged cells. Researchers have shown that DNA double-strand breaks (DSBs) are also capable of inducing monoubiquitination of H2B. This process depends upon the recruitment to DSB sites, as well as ATM-dependent phosphorylation of the RNF20-RNF40 heterodimer, thus highlighting a role for this E3 ligase in DSB repair pathways (6). Indeed, investigators have shown that loss of RNF20-RNF40 function promotes replication stress and chromosomal instability, which may constitute an early step in malignant transformation that precedes cell invasion (7).

Specificity/Sensitivity: RNF20 (D6E10) XP® Rabbit mAb recognizes endogenous levels of total RNF20 protein. This antibody recognizes a second band at 80 kDa in mouse lysates, corresponding to mouse RNF20 isoform 2. This antibody does not cross-react with RNF40 protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly516 of human RNF20 protein.

Background References:

(1) Wood, A. et al. (2003) *Mol Cell* 11, 267-74.

(2) Hwang, W.W. et al. (2003) *Mol Cell* 11, 261-6.

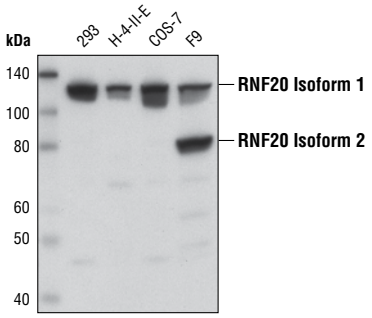
(3) Kao, C.F. et al. (2004) *Genes Dev* 18, 184-95.

(4) Kim, J. et al. (2005) *Mol Cell* 20, 759-70.

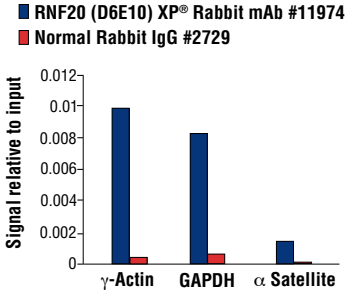
(5) Zhu, B. et al. (2005) *Mol Cell* 20, 601-11.

(6) Moyal, L. et al. (2011) *Mol Cell* 41, 529-42.

(7) Chernikova, S.B. et al. (2012) *Cancer Res*, Epub ahead of print.



Western blot analysis of extracts from various cell lines using RNF20 (D6E10) XP® Rabbit mAb.



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 HeLa cells and either 10 µl of RNF20 (D6E10) XP® Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human γ-Actin Promoter Primers #5037, SimpleChIP® Human GAPDH Promoter Primers #4471, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

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.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:200
Chromatin IP	1:50

Tween® is a registered trademark of ICI Americas, Inc.

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

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.