Revision 3

Store at -20C

#11974

RNF20 (D6E10) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, ChIP, C&R	H M R Mk	Endogenous	120	Rabbit IgG	#Q5VTR2	56254

Product Usage Information

For optimal ChIP results, use 10 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 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.

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:200
Chromatin IP	1:50
CUT&RUN	1:50

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.

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.

Species predicted to react based on 100% sequence homology

Hamster, Dog, Pig, Horse, Guinea Pig

Source / Purification

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

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).

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.

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 BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **IP:** Immunoprecipitation **ChIP:** Chromatin IP **C&R:** CUT&RUN

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat **Mk:** Monkey

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