#12187 Store at -20°C

# RNF40 (D2R20) Rabbit mAb

100 μl (10 western blots)

New 12/12

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

Applications Species Cross-Reactivity\* Molecular Wt. Isotype W, IP H 130 kDa Rabbit IgG\*\* Endogenous

Background: In mammalian cells, the significance of histone H2B ubiguitination 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:** RNF40 (D2R20) Rabbit mAb recognizes endogenous levels of total RNF40 protein. This antibody does not cross-react with RNF20/BRE1A.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp537 of human RNF40 protein.



0.1% Tween-20 at 4°C with gentle shaking, overnight.



Western blot analysis of extracts from 293T cells, mock transfected (-) or transfected with constructs expressing Myc/DDK-tagged full-length human RNF20 (hRNF20-Myc/ DDK; +) and Myc/DDK-tagged full-length human RNF40 (hRNF40-Myc/DDK; +), using RNF40 (D2R20) Rabbit mAb (upper) or DYKDDDDK Tag Antibody #2368 (lower).

 Western blot analysis of extracts from various cell lines using RNF40 (D2R20) Rabbit mAb.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS,



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### Entrez-Gene ID #9810 Swiss-Prot Acc. #075150

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/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.

<b>Recommended Antibody</b>	Dilutions:
Western Blotting	

Western Blotting	1:1000
Immunoprecipitation	1:100

# For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended complementary products.

## Background References:

(1) Wood, A. et al. (2003) *Mol. Cell* 11, 267–274.

(2) Hwang, W.W. et al. (2003) Mol. Cell 11, 261-266.

(3) Kao, C.F. et al. (2004) Genes Dev. 18, 184-195.

(4) Kim, J. et al. (2005) Mol. Cell 20, 759-770.

- (5) Zhu, B. et al. (2005) Mol. Cell 20, 601-611.
- (6) Moyal, L. et al. (2011) Mol .Cell 41, 529-542.
- (7) Chernikova, S.B. et al. (2012) *Cancer Res.*, Epub ahead of print.



Immunoprecipitation of RNF40 from 293T cell extracts, using Rabbit (DA1E) mAb IgG XP<sup>®</sup> Isotype Control #3900 (Iane 2) or RNF40 (D2R20) Rabbit mAb (Iane 3). Lane 1 is 10% input. Western blot analysis was performed using RNF40 (D2R20) Rabbit mAb.

 Applications Key:
 W—Western
 IP—Immunoprecipitation
 IHC—Immunohistochemistry
 ChIP—Chromatin Immunoprecipitation
 IF—Immunofluorescence
 F—Flow cytometry
 E-P—ELISA-Peptide

 Species Cross-Reactivity Key:
 H—human
 M—mouse
 R—rat
 Hm—hamster
 Mk—monkey
 Mi—mink
 C—chicken
 Dm—D. melanogaster
 X—zebrafish
 B—bovine

 Dg—dog
 Pg—pig
 Sc—S. cerevisiae
 AII—all species expected
 Species enclosed in parentheses are predicted to react based on 100% homology.

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