#8814 Store at -20°C

Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb

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Entrez-Gene ID #1499
UniProt Acc. #P35222

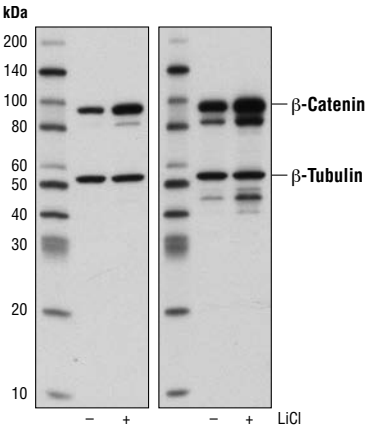
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, ChIP, F, ChIP-seq Endogenous	H, M, R, Mk, (C, X, Z, B, Dg, Pg, Hr, Guinea Pig)	92 kDa	Rabbit IgG**

Background: β-catenin is a key downstream effector in the Wnt signaling pathway (1). It is implicated in two major biological processes in vertebrates: early embryonic development (2) and tumorigenesis (3). CK1 phosphorylates β-catenin at Ser45. This phosphorylation event primes β-catenin for subsequent phosphorylation by GSK-3 (4-6). GSK-3β destabilizes β-catenin by phosphorylating it at Ser33, Ser37, and Thr41 (7). Mutations at these sites result in the stabilization of β-catenin protein levels and have been found in many tumor cell lines (8).

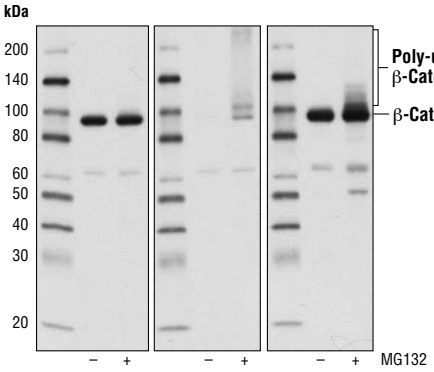
Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb is designed to specifically recognize the stabilized form of β-catenin, i.e., protein that has not been phosphorylated by GSK-3 and thus is functionally active in cell-cell adhesion and/or the canonical Wnt signaling pathway.

Specificity/Sensitivity: Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb recognizes endogenous β-catenin protein when residues Ser33, Ser37, and Thr41 are not phosphorylated. It does not detect β-catenin protein if tri-phosphorylated at Ser33/Ser37/Thr41. This antibody may also detect β-catenin protein when singly phosphorylated at Ser33. This specificity data was derived from competition ELISA and dot blot analyses using synthetic peptides.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser37 of human β-catenin protein.



Western blot analysis of extracts from 293T cells, untreated (-) or treated (+) with LiCl (20 mM, 20 hr at 37°C), using Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb (left) and β-Catenin Antibody #9562 (right). Equal protein loading was confirmed using β-Tubulin (9F3) Rabbit mAb #2128.



◀ *Western blot analysis of extracts from 293T cells, untreated (-) or treated (+) with MG132 (10 μM, 4 hr at 37°C), using Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb (left), Phospho-β-Catenin (Ser33/37/Thr41) Antibody #9561 (center), or β-Catenin (6B3) Rabbit mAb #9582 (right). Note that Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb fails to detect poly-ubiquitinated β-catenin in MG132-treated cells, indicating its specificity for stabilized protein.*

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:

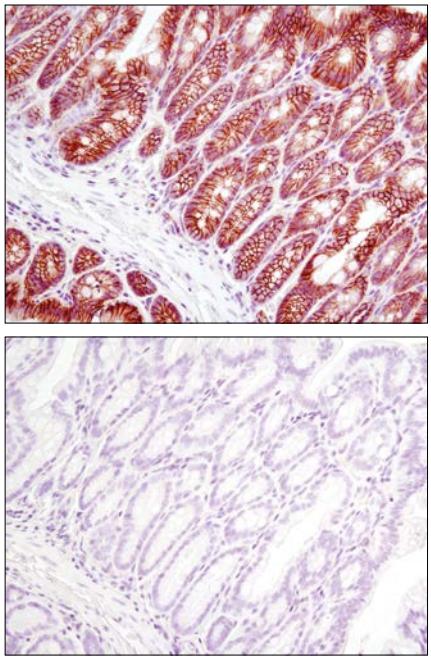
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:800
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunofluorescence (IF-IC)	1:800
Chromatin IP / Chromatin IP-seq	1:100
Optimal ChIP / ChIP-seq conditions: 5 µl of antibody & 10 µg of chromatin (4 x 10^6 cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.	
Flow Cytometry	1:800

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

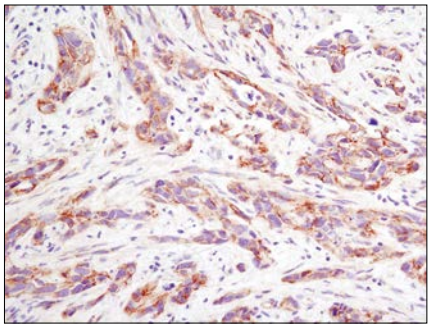
Please visit www.cellsignal.com for a complete listing of recommended companion products.

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.

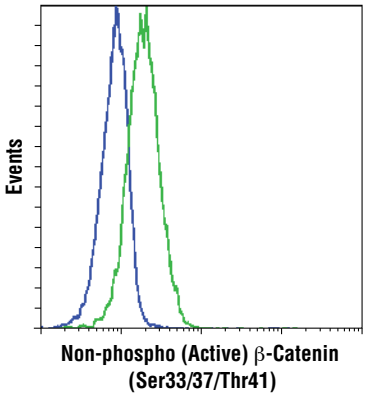
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—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.



Immunohistochemical analysis of paraffin embedded mouse colon using Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb in the presence of phospho-β-catenin (Ser33/37/Thr41) peptide (upper) or non-phospho-β-catenin (Ser33/37/Thr41) peptide (lower). Note the absence of staining only in the presence of the non-phospho-β-catenin (Ser33/37/Thr41) peptide, demonstrating non-phospho specificity.



Immunohistochemical analysis of paraffin embedded human breast carcinoma using Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb.



Flow cytometric analysis of K562 cells, untreated (blue) or treated with 6-bromoindirubin-3'-oxime (BIO) (30 nM, 40 hr) (green) using Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb.

Confocal immunofluorescent analysis of HCT-15 (upper, center; positive) or NCI-H28 (lower; negative) cells using Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb (green) in the presence of Phospho-Catenin-β(Ser33/Ser37/Thr41) peptide (upper) or Non-phospho-Catenin-β (Ser33/Ser37/Thr41) peptide (center). Red = Propidium Iodide (PI)/RNase Staining Solution #4087.

Background References:

(1) Cadigan, K.M. and Nusse, R. (1997) *Genes Dev.* 11, 3286-3305.

(2) Wodarz, A. and Nusse, R. (1998) *Annu. Rev. Cell. Dev. Biol.* 14, 59-88.

(3) Polakis, P. (1999) *Curr. Opin. Genet. Dev.* 9, 15-21.

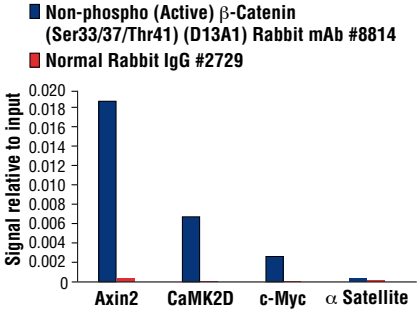
(4) Amit, S. et al. (2002) *Genes Dev.* 16, 1066-1076.

(5) Lin, C. et al. (2002) *Cell* 108, 837-847.

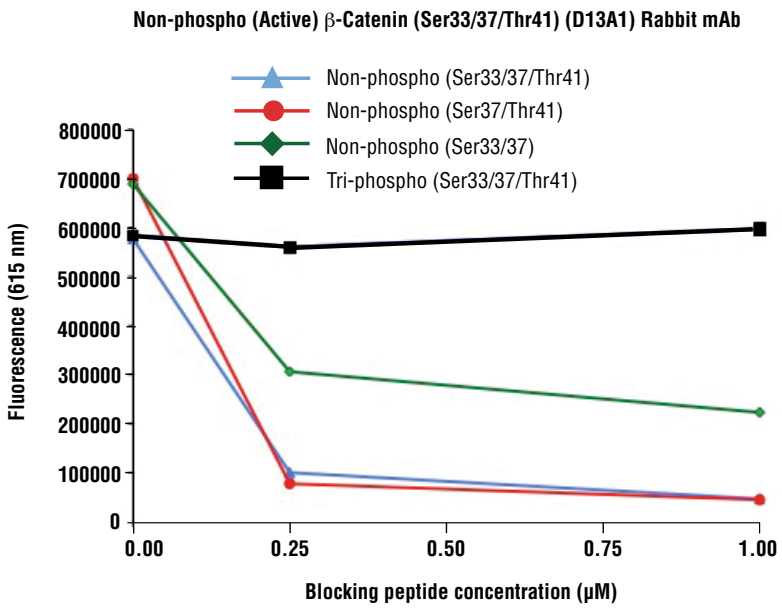
(6) Yanagawa, S. et al. (2002) *EMBO J.* 21, 1733-1742.

(7) Yost, C. et al. (1996) *Genes Dev.* 10, 1443-1454.

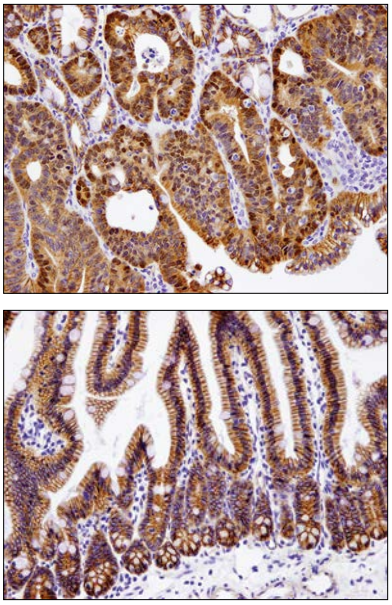
(8) Morin, P.J. (1997) *Science* 275, 1787-1790.



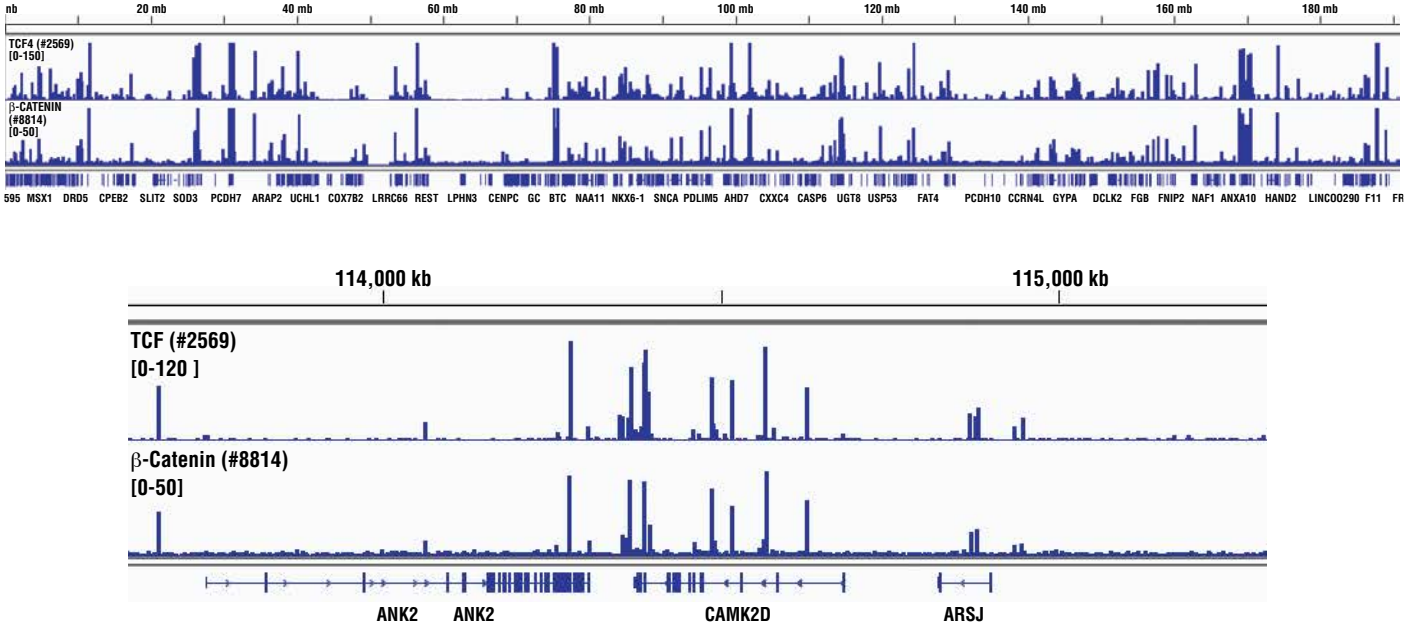
Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 HCT 116 cells and either 5 µl of Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human Axin2 Intron 1 Primers #8973, SimpleChIP® Human CaMK2D Intron 3 Primers #5111, human c-Myc promoter primers, 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.



Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb specificity was determined by peptide competition ELISA. Graph depicts binding of the antibody to non-phosphorylated β-catenin (Ser33/37/Thr41) peptide in the presence of increasing concentrations of competitor peptides. As shown, non-phosphorylated β-catenin peptides compete for binding, whereas tri-phosphorylated peptide (Ser33/37/Thr41) does not compete for binding.



Immunohistochemical analysis of paraffin-embedded Apc (Min/+) mouse intestinal adenoma (upper) and adjacent normal intestinal epithelium (lower) using Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb. Note the nuclear accumulation of active beta-Catenin in the adenoma cells.



Chromatin immunoprecipitations were performed with cross-linked chromatin from HCT116 cells and either TCF4 (C48H11) Rabbit mAb #2569 or Non-phospho (Active) β-Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. TCF4 and β-Catenin are known to associate with each other on chromatin. The figure shows binding of both TCF4 and β-Catenin across chromosome 4 (upper), including CAMK2D (lower), a known target gene of both TCF4 and β-Catenin (see additional figure containing ChIP-qPCR data).