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#13846

## p130 Cas (E1L9H) Rabbit mAb

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Entrez-Gene ID #9564

UniProt ID #P56945

rev. 09/03/19

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Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, F Endogenous	H, M, R, Mk	130 kDa	Rabbit IgG**

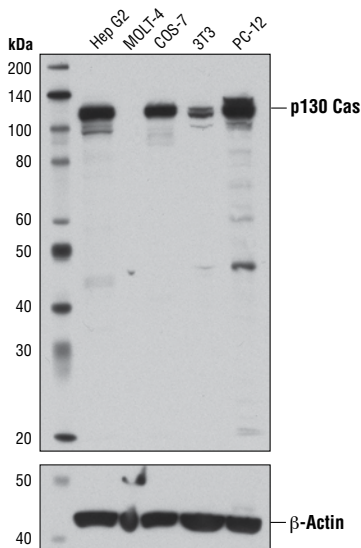
**Background:** p130 Cas (Crk-associated substrate) is a docking protein containing multiple protein-protein interaction domains. The amino-terminal SH3 domain may function as a molecular switch regulating CAS tyrosine phosphorylation, as it interacts with focal adhesion kinase (FAK) (1) and the FAK-related kinase PYK2 (2), as well as the tyrosine phosphatases PTP-1B (3) and PTP-PEST (4). The carboxy-terminal Src binding domain (SBD) contains a proline-rich motif that mediates interaction with the SH3 domains of Src-family kinases (SFKs) and a tyrosine phosphorylation site (Tyr668 and/or Tyr670) that can promote interaction with the SH2 domain of SFKs (5). The p130 Cas central substrate domain, the major region of tyrosine phosphorylation, is characterized by 15 tyrosines present in Tyr-X-X-Pro (YXXP) motifs, including Tyr165, 249, and 410. When phosphorylated, most YXXP motifs are able to serve as docking sites for proteins with SH2 or PTB domains including adaptors, C-Crk, Nck, and inositol 5'-phosphatase 2 (SHIP2) (6). The tyrosine phosphorylation of p130 Cas has been implicated as a key signaling step in integrin control of normal cellular behaviors including motility, proliferation, and survival. Aberrant Cas tyrosine phosphorylation may contribute to cell transformation by certain oncoproteins (5).

**Specificity/Sensitivity:** p130 Cas (E1L9H) Rabbit mAb recognizes endogenous levels of total p130 Cas protein.

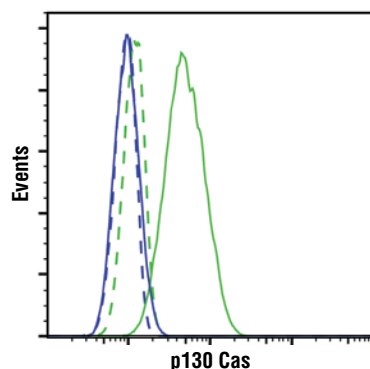
**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu116 in the SH3 domain of human p130 Cas protein.

**Background References:**

- (1) Polte, T.R. and Hanks, S.K. (1997) *J Biol Chem* 272, 5501-9.
- (2) Astier, A. et al. (1997) *J Biol Chem* 272, 228-32.
- (3) Liu, F. et al. (1996) *J Biol Chem* 271, 31290-5.
- (4) Garton, A.J. et al. (1997) *Oncogene* 15, 877-85.
- (5) Ruest, P.J. et al. (2001) *Mol Cell Biol* 21, 7641-52.
- (6) Bouton, A.H. et al. (2001) *Oncogene* 20, 6448-58.



Western blot analysis of extracts from various cell lines using p130 Cas (E1L9H) Rabbit mAb (upper) and  $\beta$ -Actin (D6A8) Rabbit mAb #8457 (lower).



Flow cytometric analysis of Jurkat cells (blue) and HT-1080 cells (green) using p130 Cas (E1L9H) Rabbit mAb (solid lines) or a concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab)<sub>2</sub> Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

**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  $-20^{\circ}\text{C}$ . 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)	1:50-1:200
<i>Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.</i>	
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
Immunofluorescence (IF-IC)	1:100-1:400
Flow Cytometry	1:100-1:400

For product specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

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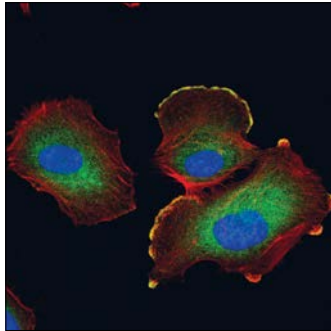
**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.**

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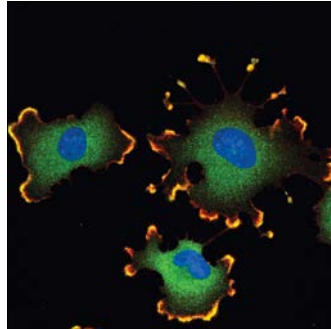
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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken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

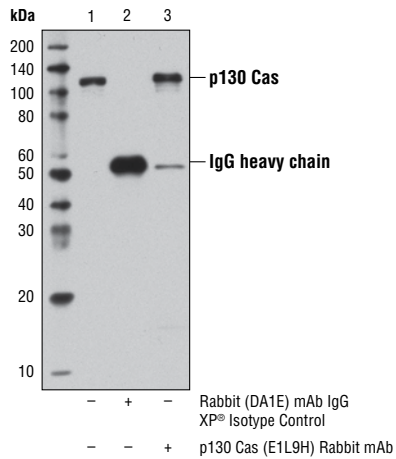
**Untreated**



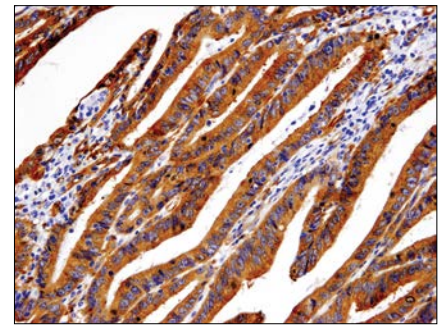
**TPA-treated**



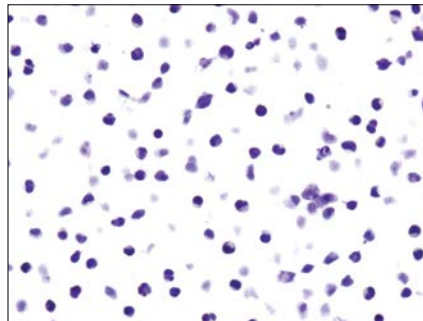
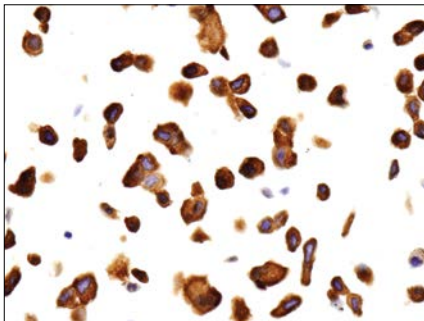
Confocal immunofluorescent analysis of SNB19 cells either untreated (upper) or treated with TPA #9905 (100nM, 1hr; lower) using p130 Cas (E1L9H) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor= DRAQ5® #4084 (fluorescent DNA dye).



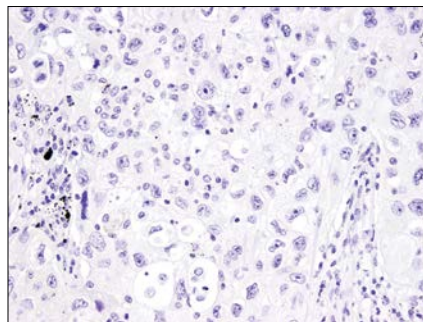
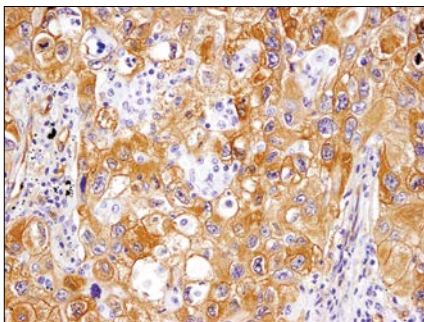
Immunoprecipitation of p130 Cas from Hep G2 cell extracts using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or p130 Cas (E1L9H) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using p130 Cas (E1L9H) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using p130 Cas (E1L9H) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded T-47D (left) and Jurkat (right) cell pellets using p130 Cas (E1L9H) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human non-small cell lung carcinoma using p130 Cas (E1L9H) Rabbit mAb in the presence of control peptide (left) and antigen-specific peptide (right).