

#2680 Store at -20°C

# p35/25 (C64B10) Rabbit mAb



✓ 100 µl  
(10 western blots)

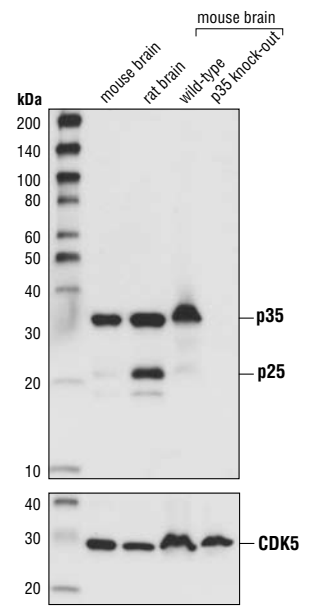
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-F Endogenous	H, M, R	25, 35 kDa	Rabbit IgG**

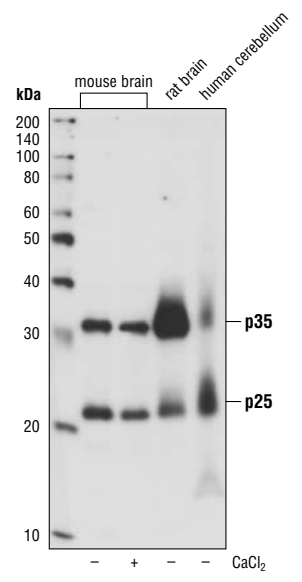
**Background:** Cyclin-dependent kinases (CDKs) are serine/threonine kinases that are activated by cyclins and govern eukaryotic cell cycle progression. While CDK5 shares high sequence homology with its family members, it is thought mainly to function in postmitotic neurons, regulating the cytoarchitecture of these cells. Analogous to cyclins, p35 and p39 associate with and activate CDK5 despite the lack of sequence homology. CDK5 is ubiquitously expressed, but high levels of kinase activity are detected primarily in the nervous system due to the narrow expression pattern of p35 and p39 in post-mitotic neurons. A large number of CDK5 substrates have been identified although no discrete substrates have been attributed as a function of p35 vs. p39. Amongst many, substrates of CDK5 include p35 and p39. p35 is rapidly degraded (T1/2 <20 min) by the ubiquitin-proteasome pathway (1). However, p35 stability increases as CDK5 kinase activity decreases, and this is likely a result of decreased phosphorylation of p35 at Thr138 by CDK5 (2). NGF activates Erk and EGR1, and induces p35 expression in PC12 cells (3). Proteolytic cleavage of p35 by calpain produces p25 upon neurotoxic insult, resulting in prolonged activation of CDK5 by p25. Accumulation of p25 is found in neurodegenerative diseases such as Alzheimer's disease and Amyotrophic Lateral Sclerosis (ALS) (4–5).



Western blot analysis of extracts from mouse and rat brain and wild-type mouse and p35 knock-out mouse brain using p35/25 (C64B10) Rabbit mAb (upper) and CDK5 Antibody #2506 (lower). Matching wild-type and p35 knock-out mouse brain was kindly provided by Dr. Li-Huei Tsai, Massachusetts Institute of Technology, Cambridge, MA.

**Specificity/Sensitivity:** p35 Antibody detects endogenous levels of total p35 protein. The antibody also detects endogenous p25 resulting from calpain-mediated cleavage upon neurotoxic insult.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide from the carboxy terminus of human p35.



◀ Western blot analysis of extracts from mouse brain, rat brain and human cerebellum using p35/p25 (C64B10) Rabbit mAb.

Entrez-Gene ID #8851  
Swiss-Prot Acc. #Q15078

**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	1:50
Immunohistochemistry (Paraffin)	1:100†
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-F)	1:200

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

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

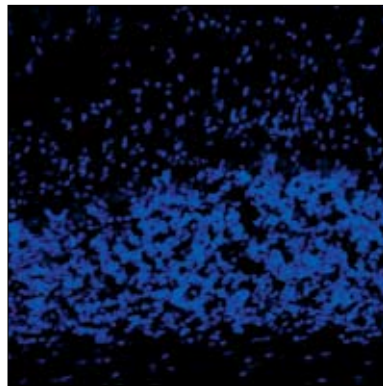
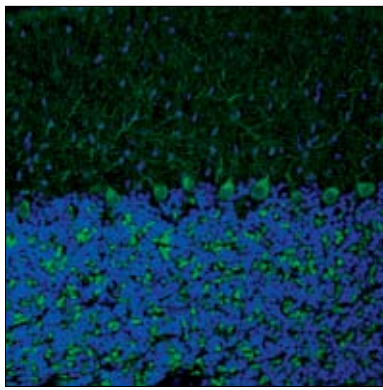
- Background References:**
- (1) Dhavan, R. and Tsai, L.H. (2001) *Nat. Rev. Mol. Cell Biol.* 2, 749–759.
  - (2) Patrick, G.N. et al. (1998) *J. Biol. Chem.* 273, 24057–24064.
  - (3) Harada, T. et al. (2001) *Nat. Cell Biol.* 3, 453–459.
  - (4) Lee, M.S. et al. (2000) *Nature* 405, 360–364.
  - (5) Kusakawa, G. et al. (2000) *J. Biol. Chem.* 275, 17166–17172.

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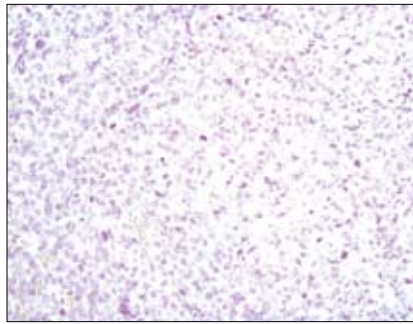
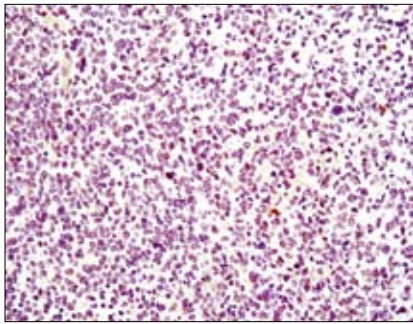
**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

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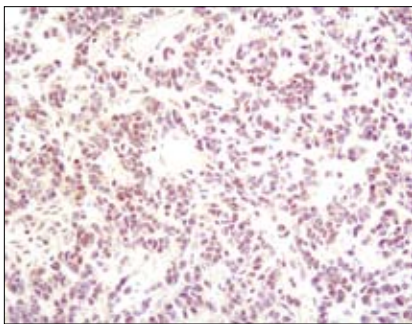
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish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



*Confocal immunofluorescent analysis of mouse cerebellum from normal (left) or p35 knockout (right) mice using p35/25 (C64B10) Rabbit mAb (green). Blue pseudocolor =DRAQ5® #4084 (fluorescent DNA dye).*



*Immunohistochemical analysis of paraffin-embedded human astrocytoma using p35/25 (C64B10) Rabbit mAb #2680 in the presence of control peptide (left) or antigen specific peptide (right).*



*Immunohistochemical analysis of paraffin embedded human meningioma using p35/25 (C64B10) Rabbit mAb #2680.*