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ProductInformation

Anti-phospho-Tyrosine Hydroxylase (pSer^{40})
Developed in Rabbit, Affinity Isolated Antibody

Product Number **T 9573**

Product Description

Anti-phospho-Tyrosine Hydroxylase (TH) (pSer^{40}) is developed in rabbit using a synthetic phosphorylated peptide derived from the region of rat TH that is phosphorylated on serine 40 as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards a non-phosphorylated TH.

The antibody specifically recognizes rat TH phosphorylated at serine 40 (60 kDa). Human, porcine, quail, mouse, and non-human primate have 100% amino acid sequence identity with the antigen used to raise the antibody It has been used in immunoblotting, immunocytochemistry, and immunofluorescence applications.

Tyrosine hydroxylase (TH) is involved in the conversion of phenylalanine to dopamine. Tyrosine hydroxylase catalyzes the initial, rate-limiting step of the catecholamine biosynthetic pathway. Catecholamines include dopamine, noradrenaline, and adrenaline. These three catecholamines are important neurotransmitters and hormones that regulate visceral functions, motor coordination, and arousal in adults. In rodent embryos, the TH gene becomes transcriptionally active in developing neuroblasts during midgestation, before the onset of neurotransmission.[1]

Tyrosine hydroxylase is activated by phosphorylation. Recombinant human tyrosine hydroxylase (hTH1) is phosphorylated by mitogen and stress-activated protein kinase 1 (MSK1) at serine 40 and by p38 regulated/activated kinase (PRAK) on serine 19. Phosphorylation of both Ser40 and Ser19 induces a high-affinity binding of 14-3-3 proteins that inhibits the rate of dephosphorylation of Ser19 and Ser40 by 82% and 36%, respectively.[1] The phosphorylation of hTH1 on Ser19 caused a threefold increase in the rate of phosphorylation of Ser40. These studies provide new insights into the possible roles of stress-activated protein kinases in the regulation of catecholamine biosynthesis.[1]

Studies of the protein kinases and protein phosphatases that act on tyrosine hydroxylase were performed on bovine adrenal chromaffin cells in the presence of calcium or various kinase or phosphatase inhibitors. Ca^{2+} increased the phosphorylation of Ser19 and Ser40. Cyclic AMP and phorbol dibutyrate in the presence of calcium increased the phosphorylation of only serine 40. Serine 31 and serine 8 were not phosphorylated. The Ca^{2+}-stimulated phosphorylation of serine 40 was reduced by specific inhibitors of protein kinase A (56% with H89 and 38% with PKAi 5-22 amide) and protein kinase C (70% with Ro 31-8220 and 54% with PKCi 19-31), suggesting that protein kinases A and C contributed to most of the phosphorylation of this site. Results with okadaic acid and microcystin suggested that Ser19 and Ser40 were dephosphorylated by PP2A.[2]

Leptin appears to play a role in TH phosphorylation. Leptin (3-30 nM) causes a significant increase in [^{14}C]-catecholamine synthesis from [^{14}C]- tyrosine, but not from [^{14}C]-DOPA. Incubation of cells with leptin results in activation and phosphorylation of tyrosine hydroxylase. Leptin induces a transient activation of mitogen-activated protein kinases (MAPKs). U0126, an inhibitor of MAPK kinase, abolishes the effect of leptin on [^{14}C]-catecholamine synthesis. These findings suggest that leptin leads to phosphorylation and activation of tyrosine hydroxylase and subsequently stimulates catecholamine synthesis through MAPK and Ca^{2+} pathways in the adrenal medulla.[3]

Reagent

The antibody is supplied as a solution in 10 mM HEPES, pH 7.5, 150 mM NaCl, 100 µg/ml BSA and 50% glycerol.

Storage/Stability
Store at –20 °C. For extended storage, upon initial thawing, freeze in working aliquots. Due to the high viscosity of glycerol, mix well before aliquoting. Do not store in frost-free freezers. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

Product Profile
The supplied reagent is sufficient for 10 immunoblots.

A recommended working dilution of 1:1,000 is determined by immunoblotting using rat pheochromocytoma PC-12 cells. A band of 60 kDa is detected. Some higher molecular weight bands may be detected depending on brain region being studied, protein loads, and detection methods. The same working dilution is recommended for all other applications.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

1. Toska, K., et al., Regulation of tyrosine hydroxylase by stress-activated protein kinases., J. Neurochem., **83**, 775-783 (2002).
2. Goncalves C. A., et al., Tyrosine hydroxylase phosphorylation in digitonin-permeabilized bovine adrenal chromaffin cells: the effect of protein kinase and phosphatase inhibitors on Ser19 and Ser40 phosphorylation. J. Neurochem., **69**, 2387-2396 (1997).
3. Shibuya I., et al., Regulation of catecholamine synthesis by leptin., Ann. N. Y. Acad. Sci., **971**, 522-527 (2002).

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