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ProductInformation

Anti-phospho-Tyrosine Hydroxylase-[pSer^{31}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **P 1747**

Product Description

Anti-phospho-Tyrosine Hydroxylase-[pSer^{31}] is developed in rabbit using a synthetic phosphopeptide corresponding to amino acid residues surrounding the serine 31 of rat tyrosine hydroxylase (TH) as immunogen. The antiserum is affinity purified using sequential chromatography on phospho- and nonphospho-peptide affinity columns.

The antibody detects most mammalian species. It has been used in immunoblotting, ELISA, immunocytochemistry, and immunoprecipitation 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]

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

Anti-phospho-Tyrosine Hydroxylase-[pSer^{31}] is provided in 10 mM HEPES, pH 7.5, 150 mM NaCl, 100 µg/ml BSA and 50% glycerol

Storage/Stability

Store at –20 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

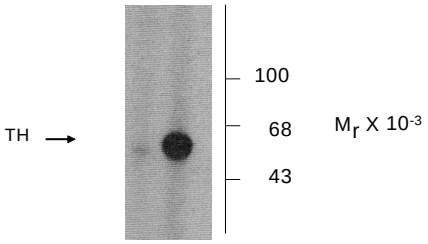
The supplied reagent is sufficient for 10 blots.

A recommended working dilution of 1:1000 is determined by immunoblotting in lysates of PC-12 cells stimulated by okadaic acid. 1:1000 working dilution is recommended for dot blot, immunohistochemistry and immunofluorescence.

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

Results

Anti-Phospho Ser[31] Tyrosine Hydroxylase



Immunoblot of PC-12 cells that had been incubated in the absence or presence of Okadaic Acid. Labeling by the anti-phospho Ser[31] TH antibody is seen only in the sample stimulated with okadaic acid (1 µM for 60 minutes).

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).
4. Witkovsky, P., et al., Influence of light and neural circuitry on tyrosine hydroxylase phosphorylation in the rat retina, J. Chem. Neuroanat., **19,** 105 – 116 (2000).

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