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### Antibodies to GTP Cyclohydrolase (Rate Limiting Enzyme for Tetrahydropterin Synthesis)

#### GTP cyclohydrolase I (GTPCHI) antibodies (Cat # GTPCHI-101AP and GTPCHI-112AP)

**G**uanosine triphosphate cyclohydrolase I (GTPCHI) is the rate limiting enzyme for the synthesis of tetrahydrobiopterin, a cofactor for hydroxylation reaction in several pathways. GTPCHI is a critical enzyme in catecholamine function and is rate limiting for the synthesis of the catecholamine co-factor tetrahydrobiopterin (6BH)4, a key cofactor necessary for nitric oxide synthase and for the hydroxylases that are involved in the production of catecholamines and serotonin. (6R)-L-Erythro 5,6,7,8 tetrahydrobiopterin (6BH(4)) is crucial in the hydroxylation of L-phenylalanine-, L-tyrosine-, and L-tryptophan-regulating catecholamine and serotonin synthesis as well as tyrosinase in melanogenesis. The GTPCHI feed back regulatory protein (GFRP) binds to GTPCHI in presence of phenylalanine and inhibit its activity in the presence of biopterin. The shear stress in vasculature caused an up-regulation of eNOS and GTPCHI accompanied by increased blood flow. The GTPCHI increase caused a rise in BH4 production which required for sustained vasorelaxant response that increases the blood flow. The cofactor supply of (6BH)4 is indeed controlled by GFRP and GTPCHI in epidermal keratinocytes and melanocytes for the biosynthesis of catecholamine and serotonin.

GTPCHI is a 241 aa (30 kDa) cytosolic protein that interact with GFRP. The expression of GTPCHI is reduced in nigrostriatal neurons in middle and aged rats, monkeys and humans. The age related decrease in GTPCHI nigral neurons suggest a phenotypic-down regulation of this enzyme rather than generalized necrosis of cells and may be responsible for the generalized age-related decrease in dopaminergic tone with nigrostriatal system (1). Gene therapy approach for Parkinsonian condition was tested using purified adeno-associated virus containing tyrosine hydroxylase (TH) and GTPCHI to infect 6-OH denervated rat striatum. The viral delivery system indeed showed >90% of viral infected neurons to be TH positive (2), however, the production of L-DOPA was only evident in transfections with TH and GTPCHI, suggesting that the trans-gene expression of TH and GTPCHI can be used to successfully deliver L-DOPA to striatum (2). The activity of GTPCHI is reduced by 50% in CNS of rats exhibiting chronic hepatic encephalopathy (3).

The GTPCHI-selective antibodies were generated against 2 peptides form unique regions on the GTPCHI protein. FabGennix Inc. has generated epitope specific rabbit anti-GTPCHI polyclonal (epitope-specific) antibodies utilizing linear and cyclic peptide sequences. The GTPCHI antibodies are affinity purified over immobilized antigen based chromatography, and the purified immunoglobulin are stabilized in antibody stabilization buffer. Limited quantities of the antigenic blocking peptide for GTPCHI antibodies are also available (inquire for availability). *FabGennix Inc.* will also conjugate antibodies with enzymes or fluorescent probes as custom service upon request at a reasonable cost.

Catalog #	Host	Description	Antigen/ control	Cross reactivity	Price
GTPCHI-101AP	Rabbit	GTPCHI affinity purified N-terminal Antibodies	Affinity purified Antibody	R, M, H	\$235
GTPCHI-112AP	Rabbit	GTPCHI affinity purified C-terminal Antibodies	Affinity purified Antibody	R, M, H	\$235
P-GTPCHI1	n/a	Antigenic blocking peptide for GTPCHI-101AP	Purified peptide	250 ug	\$125
P-GTPCHI2	n/a	Antigenic blocking peptide for GTPCHI-112AP	Purified peptide	250 ug	\$125
PC-GTPCHI	n/a	Western blot positive control for GTPCHI	Partially purified GTPCHI	For 5 applications	\$185

R = rat; M = mouse; H = humans; R = rabbit \* Actual volume is 103-110 µl; WB, Western Blot analyses; IMM, Immunoprecipitation; IHC, Immunohistochemistry, n.d, not determine.

**Immunogen:** Synthetic peptides taken form unique regions on the protein GTPCHI. Peptide I (aa 9-22; ctn gfs ere lpr pg) and peptide II (aa 28-45; eks rpp eak gaq pad awk c). Peptides were post-synthetically modified to achieve desired antigenicity using our cyclic peptide methodology. The peptides were then coupled to KLH using hetero-bifunctional cross linker for immunogen preparation.

**Concentration:** GTPCHI-101AP = IgG concentration 0.68-0.98 mg/ml; GTPCHI-112AP = IgG concentration -73-1.01mg/ml.

**Applications:** ELISA: Antibody dilution 1:20,000 for ELISA or DOT blot assay. W.B: Antibody dilution 1:500-750 for WB using PC-GTPCHI; IMM: n.d; IHC n.d. The cross species reactivity of this antibody has not been examined in detail.

**Reactivity** The antibodies GTPCHI-101AP and GTPCHI-112AP label 33-45kDa protein in PC-GTPCHI samples. In rat heart the antibodies label 3 proteins including a 33 kDa band.

**Protocols:** Standard protocol for various applications (Western blot; immunoprecipitation and immunohistochemistry) of this antibody can be obtained by calling Technical support line, general information on this antibody is provided with the product specification sheet, and however, FabGennix Inc. recommends investigators to optimize conditions.

**Form/Storage:** The antiserum is supplied in antibody stabilization buffer with preservatives. For long-term storage of antibody, store at -20°C FabGennix Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi-use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45µm filter after every use for long-term storage.

**\*Note:** Briefly centrifuge to collect liquid before opening the vial, heat the PC-GTPCHI tube in 90oC water bath for 1-2 minutes to dissolve any precipitate before use. This product is "ready-to-use" for electrophoresis. After thawing store at room temperature, Repeated freezing and thawing may result in appearance of higher molecular weight immunoreactive bands.

Western Blot of GTPCHI-101 and GTPCHI-112AP with PC-GTPCHI sample. Antibody dilution 1:750 in antibody dilution buffer (DiluOBuffer). GTPCHI approximate MW is a 31 kDa protein.

**Note:** Now you can recycle your western blots (nitrocellulose, supported membranes and PVDF membranes) by using our StripOBuffer (Cat # FGI-1989). Each stripping is guaranteed to give better signal (up to 8 stripping). No strong pungent smell of reducing agents or heating is required.

#### References:

- Chen EY, Kallwitz E, Leff SE, Cochran EJ, Mufson EJ, Kordower JH, Mandel RJ. Age-related decreases in GTP-cyclohydrolase-I immunoreactive neurons in the monkey and human substantia nigra. *J Comp Neurol.* 2000 Oct 30;426(4):534-48.
- Mandel RJ, Rendahl KG, Spratt SK, Snyder RO, Cohen LK, Leff SE. Characterization of intrastratial recombinant adeno-associated virus-mediated gene transfer of human tyrosine hydroxylase and human GTP-cyclohydrolase I in a rat model of Parkinson's disease. *J Neurosci.* 1998 Jun 1;18(11):4271-84.
- PB, Werner ER, Apelqvist G, Bugge M, Wachter H, Bengtsson F Bergqvist. Brain biopterin metabolism in chronic experimental hepatic encephalopathy. *Metab Brain Dis.* 1995 Jun;10(2):143-57.

\* For users who may require large amounts of GTPCHI-101AP and GTPCHI-112AP, please enquire about bulk material discounts.  
This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

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