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Data Sheet

TPH1 Inhibitor Screening Assay Kit

Catalog: #72056
Size: 384 reactions

BACKGROUND: Tryptophan 5-hydroxylase 1 and 2 (TPH1 and TPH2) are enzymes that catalyze the monooxygenation of tryptophan to 5-hydroxytryptophan (5-HTP) which is subsequently converted to serotonin. By catalyzing the rate-limiting step in the biosynthesis of serotonin, these enzymes also play key roles in the signaling of other neurotransmitters downstream of serotonin synthesis, like melatonin. Additionally, TPH enzymes may play a role in immune regulation through tryptophan depletion.

DESCRIPTION: The TPH1 Inhibitor Screening Assay Kit is designed to measure enzyme inhibition in a 384 reaction format. This fluorescence based assay kit is especially suitable for high throughput screening applications. The procedure is straightforward and simple, requiring only the mixing of two solutions, incubation, addition of quench solution, and measurement of fluorescence.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71192	TPH1	2 x 50 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	TPH Enzyme Solution	10 ml	-80°C	
	TPH Reaction Solution	12 ml	-80°C	
	TPH Quench Solution	3 x 1 ml	-20°C	
	Black, 384 Well Plate	1	Room Temp	

MATERIALS REQUIRED BUT NOT SUPPLIED:
Microplate reader capable of measuring Fluorescence

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: At least 6 months from date of receipt when stored as directed.

REFERENCE:

1. Moran G. R., et al. J. Biol. Chem. 1998; 273:12259-12266.
2. Nowak E. C., et al., J. Exp. Med. 2012; 209:2127-2135.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

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1) Thaw **TPH Enzyme Solution** on ice. Take only a sufficient quantity needed for the assay. Keep solution on ice. Store remaining solution in aliquots at -80°C.
2) Thaw **TPH Reaction Solution** on ice. Take only a sufficient quantity needed for the assay. Keep solution on ice. Store remaining solution in aliquots at -80°C.

3) Keep plate cold on ice. Add 5 µl of inhibitor solution to each well labeled "Test Inhibitor". Add 5 µl of the same solution without inhibitor (Inhibitor Buffer) to each well labeled "Negative Control" and "Positive Control".

	Negative Control*	Positive Control	Test Inhibitor
Test Inhibitor	–	–	5 µl
Inhibitor Buffer (no inhibitor)	5 µl	5 µl	–
Enzyme Solution	20 µl	–	–
TPH1 Solution (11 ng/µl)	–	20 µl	20 µl
Reaction Solution	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

4) Add 20 µl of **TPH Enzyme Solution** to each well labeled "Negative Control".

5) Thaw **TPH1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **TPH1** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: **TPH1** is very sensitive to freeze/thaw cycles. Do not reuse thawed aliquots or diluted enzyme.

6) Dilute **TPH1** to 11 ng/µl (220 ng/reaction) in **TPH Enzyme Solution**. Keep on ice until ready to use.

7) Add 20 µl of diluted **TPH1** to each well labeled "Positive Control" and "Test inhibitor".

8) Initiate reaction by adding 25 µl of **TPH Reaction Solution** to each well. Remove plate from ice and incubate for 4 hours at 4ºC.

9) After incubation, add 5 µl of **TPH Quench Solution** to each well. After quenching, plate can be handled at room temperature.

10) Read the fluorescent intensity in a microtiter-plate reader.

Instrument Settings

Reading Mode	λ (nm)
Excitation Wavelength	300
Emission Wavelength	360

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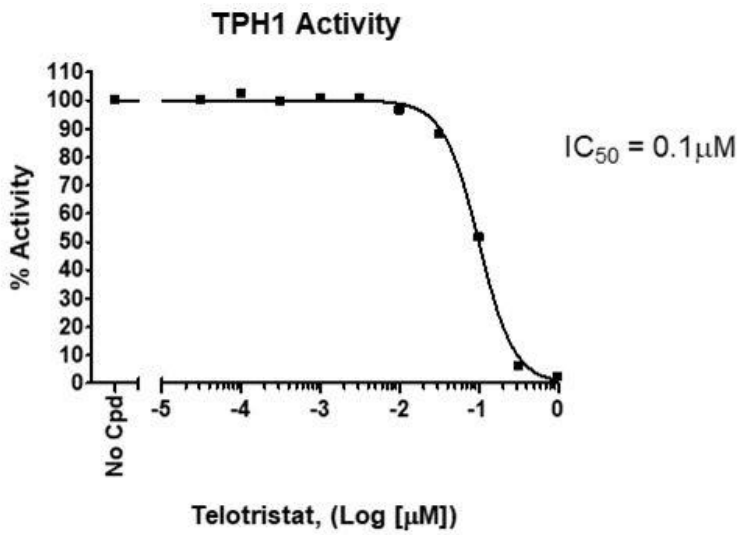
CALCULATING RESULTS:
Subtract average "Negative Control" value from average "Positive Control" value to obtain total Δ Fluorescence. Subtract average "Negative Control" value from each "Test Inhibitor" value to obtain Δ Fluorescence of test compounds.

EXAMPLE OF ASSAY RESULTS:



Inhibition of TPH1 by Telotristat, measured using the *TPH1 Assay Kit*, BPS Bioscience # 72056. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

Product	Catalog #	Size
TPH1, His-tag	71192	50 µg
TPH2, His-tag	71193	50 µg
TPH1 Inhibitor Screening Assay Kit	72053	96 rxns.
TPH2 Inhibitor Screening Assay Kit	72054	96 rxns.
TPH2 Inhibitor Screening Assay Kit	72057	384 rxns.
Human TDO, His-tag	71195	50 µg
Mouse TDO, His-tag	71241	50 µg
hTDO-HEK293 Recombinant Cell line	60534	2 vials
TDO Cellular Activity QuickDetect™ Supplements	62002-1	100 rxns.
Human TDO Inhibitor Screening Assay Kit	72023	96 rxns.