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**Data Sheet**  
**TPH2 Inhibitor Screening Assay Kit**  
Catalog: #72057  
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 TPH2 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   |                                    |
|-----------|-----------------------|----------|-----------|------------------------------------|
| 71193     | TPH2                  | 30 µg    | -80°C     | <b>(Avoid freeze/thaw cycles!)</b> |
|           | TPH Enzyme Solution   | 2 x 5 ml | -80°C     |                                    |
|           | TPH Reaction Solution | 2 x 6 ml | -80°C     |                                    |
|           | TPH Quench Solution   | 3 x 1 ml | -20°C     |                                    |
| 79961     | Black, 384 Well Plate |          | 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.**

- 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.

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- 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             | —                | —              |
| TPH2 Solution (6 ng/µl)         | —                 | 20 µl            | 20 µl          |
| Reaction Solution               | 25 µl             | 25 µl            | 25 µl          |
| <b>Total</b>                    | <b>50 µl</b>      | <b>50 µl</b>     | <b>50 µl</b>   |

- 4) Add 20 µl of **TPH Enzyme Solution** to each well labeled “Negative Control”.
- 5) Thaw **TPH2** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **TPH2** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: **TPH2** is very sensitive to freeze/thaw cycles. Do not reuse thawed aliquots or diluted enzyme.
- 6) Dilute **TPH2** to 6 ng/µl (120 ng/reaction) in **TPH Enzyme Solution**. Keep on ice until ready to use.
- 7) Add 20 µl of diluted **TPH2** 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 4°C.
- 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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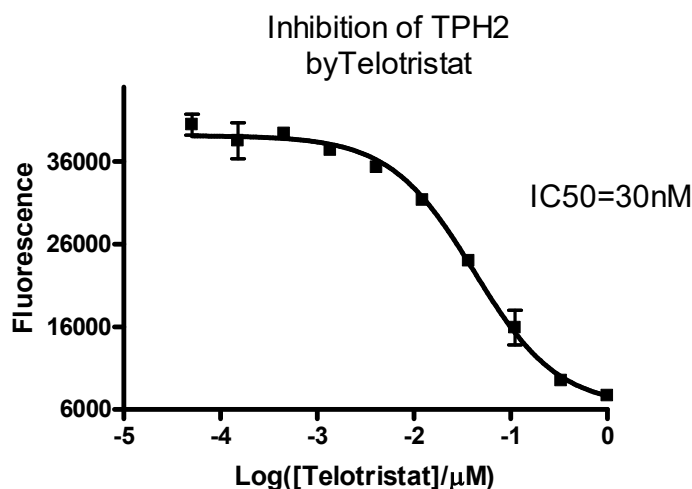


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### CALCULATING RESULTS:

Subtract average "Negative Control" value from average "Positive Control" value to obtain total  $\Delta$  Fluorescence. Subtract average "Negative Control" value from each "Test Inhibitor" value to obtain  $\Delta$  Fluorescence of test compounds.

### EXAMPLE OF ASSAY RESULTS:



Inhibition of TPH2 by Telotristat, measured using the *TPH1 Assay Kit*, BPS Bioscience # 72057. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

### RELATED PRODUCTS:

| <u>Product</u>                                 | <u>Catalog #</u> | <u>Size</u> |
|--|------------------|-------------|
| TPH1, His-tag                                  | 71192            | 50 $\mu$ g  |
| TPH2, His-tag                                  | 71193            | 50 $\mu$ g  |
| TPH1 Inhibitor Screening Assay Kit             | 72053            | 96 rxns.    |
| TPH2 Inhibitor Screening Assay Kit             | 72054            | 96 rxns.    |
| TPH1 Inhibitor Screening Assay Kit             | 72056            | 384 rxns.   |
| Human TDO, His-tag                             | 71195            | 50 $\mu$ g  |
| Mouse TDO, His-tag                             | 71241            | 50 $\mu$ g  |
| 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.    |

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