

## EnzyFluo™ Tryptophan Assay Kit (ETRP-100)

### Quantitative Fluorimetric Tryptophan Determination

#### DESCRIPTION

**TRYPTOPHAN** is one of the eight essential amino acids that the body cannot synthesize and must be obtained through diet. Tryptophan is the biochemical precursor to the neurotransmitter serotonin, which has important roles in biological processes such as regulation of appetite, sleep, and mood. Imbalances of serotonin have been linked to numerous mental health disorders. Tryptophan is also a precursor to the neurotransmitter melatonin, which is heavily involved in regulating the body's sleep cycle. BioAssay Systems' EnzyFluo™ tryptophan assay uses a coupled enzymatic reaction to determine the tryptophan concentration of a sample with the addition of a single working reagent. The fluorescence intensity at  $\lambda_{ex/em} = 530/585$  nm is directly proportional to tryptophan concentration in the sample.

#### KEY FEATURES

**Fast and sensitive.** Linear detection range: 10 to 400  $\mu$ M tryptophan.

**Convenient and high-throughput.** Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

#### APPLICATIONS

Tryptophan determination in serum.

#### KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

**Enzyme Mix:** 12 mL    **TRP Enzyme:** 120  $\mu$ L  
**Dye Reagent:** 120  $\mu$ L    **Tryptophan Standard (5 mM):** 100  $\mu$ L

**Storage conditions.** The kit is shipped on ice. Store all kit components at -20 °C. Shelf life of six months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

#### PROCEDURES

- Samples.** Samples require an internal standard and need three separate reactions: 1) sample plus standard, 2) sample alone and 3) sample blank. For the internal standard prepare 500  $\mu$ L of 100  $\mu$ M tryptophan standard by mixing 10  $\mu$ L 5 mM Standard and 490  $\mu$ L dH<sub>2</sub>O. For the sample plus standard well, add 5  $\mu$ L 100  $\mu$ M tryptophan and 10  $\mu$ L sample. For the sample and sample blank wells, add 5  $\mu$ L dH<sub>2</sub>O and 10  $\mu$ L sample.
- Tryptophan Detection.** Prepare enough working reagent (WR) for all samples plus standards and samples alone. For each reaction combine the following: 105  $\mu$ L Enzyme Mix, 1  $\mu$ L Dye Reagent, and 1  $\mu$ L TRP Enzyme. For the Sample Blanks, prepare a blank working reagent (BWR) **without** the TRP Enzyme. Add 100  $\mu$ L of WR to each sample plus standard and sample alone well. Add 100  $\mu$ L BWR to each sample blank well. Tap plate to mix briefly and thoroughly. Incubate plate protected from light for 30 min at RT.
- Read fluorescence at  $\lambda_{ex/em} = 530/585$  nm.

#### CALCULATION

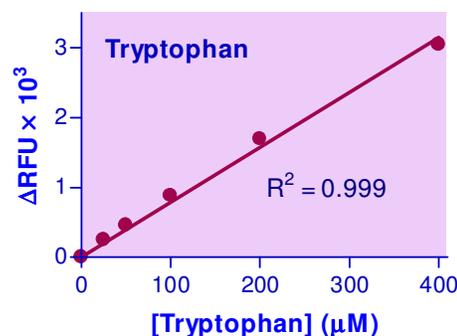
The sample tryptophan concentration is computed as follows:

$$[\text{Tryptophan}] = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{F_{\text{STANDARD}} - F_{\text{SAMPLE}}} \times \frac{[\text{Standard}]}{2} \times n \text{ (}\mu\text{M)}$$

$$= \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{F_{\text{STANDARD}} - F_{\text{SAMPLE}}} \times 50 \times n \text{ (}\mu\text{M)}$$

where  $F_{\text{SAMPLE}}$ ,  $F_{\text{BLANK}}$  and  $F_{\text{STANDARD}}$  are the fluorescence readings of the Sample, Sample Blank, and the Sample plus Standard respectively.  $n$  is the sample dilution factor. **Notes:** The volume of the internal standard is 2 $\times$  lower than the sample volume (5  $\mu$ L standard : 10  $\mu$ L sample); thus, the internal standard concentration should be divided by 2. If the calculated tryptophan concentration is >400  $\mu$ M, dilute sample in dH<sub>2</sub>O and repeat assay. Multiply result by the dilution factor  $n$ .

**Conversions:** 1  $\mu$ M tryptophan equals 0.204 mg/L, 0.0020% or 0.204 ppm.



Tryptophan spikes in bovine serum

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting (multi-channel) devices. Black, flat bottom 96-well plates (e.g. VWR cat# 89089-582), and fluorescent plate reader capable of reading at  $\lambda_{ex/em} = 530/585$  nm.

#### LITERATURE

- Hoshino, Y et al (1984). Blood Serotonin and Free Tryptophan Concentration in Autistic Children. *Neuropsychobiology* 11:22-27.
- Yunus, MB et al (1992). Plasma tryptophan and other amino acids in primary fibromyalgia: a controlled study. *J Rheumatol* 19(1): 90-94.
- Niskanen, P et al (1976). The daily rhythm of plasma tryptophan and tyrosine in depression. *Br J Psychiatry*.128(1): 67-73.