

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 $\lambda_{\text{ex/em}} = 530/585$ nm is directly proportional to tryptophan concentration in the sample.

KEY FEATURES

Fast and sensitive. Linear detection range: 10 to 400 μM 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 μL
Dye Reagent: 120 μL **Tryptophan Standard (5 mM):** 100 μL

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 μL of 100 μM tryptophan standard by mixing 10 μL 5 mM Standard and 490 μL dH₂O. For the sample plus standard well, add 5 μL 100 μM tryptophan and 10 μL sample. For the sample and sample blank wells, add 5 μL dH₂O and 10 μL sample.
- Tryptophan Detection.** Prepare enough working reagent (WR) for all samples plus standards and samples alone. For each reaction combine the following: 105 μL Enzyme Mix, 1 μL Dye Reagent, and 1 μL TRP Enzyme. For the Sample Blanks, prepare a blank working reagent (BWR) **without** the TRP Enzyme. Add 100 μL of WR to each sample plus standard and sample alone well. Add 100 μL 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 $\lambda_{\text{ex/em}} = 530/585$ nm.

CALCULATION

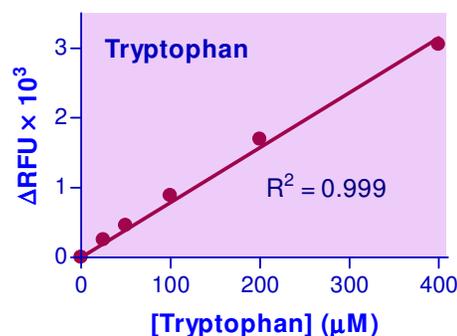
The sample tryptophan concentration is computed as follows:

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

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

where F_{SAMPLE} , F_{BLANK} and F_{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 μL standard : 10 μL sample); thus, the internal standard concentration should be divided by 2. If the calculated tryptophan concentration is >250 μM , dilute sample in dH₂O and repeat assay. Multiply result by the dilution factor n .

Conversions: 1 μM 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 $\lambda_{\text{ex/em}} = 530/585$ 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.

