Tryptophan Assay Kit (Fluorometric)

LS-K335-100 (100 Tests) • **Store at -20°C**

Introduction

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. LSBio's 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 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.

Components

Component	K335-100
	100 Tests
Enzyme Mix	12 mL
Dye Reagent	120 µL
TRP Enzyme	120 µL
Tryptophan Standard (5 mM)	100 µL

Materials Not Supplied

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.

Storage

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

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Assay Procedure

Procedure

1. 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 dH_2O. 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 dH_2O and 10 µL sample.
2. 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.
3. Read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

Calculations

The sample tryptophan concentration is computed as follows:

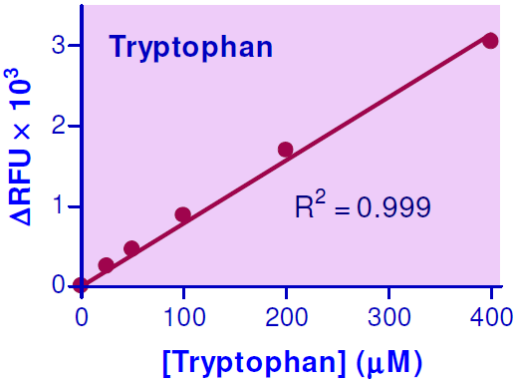
$$[\text{Tryptophan}] = \frac{F_{SAMPLE} - F_{BLANK}}{F_{STANDARD} - F_{SAMPLE}} \times \frac{[\text{Standard}]}{2} \times n \quad (\mu M)$$

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

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× 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 >400 µM, dilute sample in dH_2O 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.

Sample Data



Tryptophan spikes in bovine serum

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