

Technical Bulletin

Tryptophan Assay Kit

Catalogue number **MAK523**

Product 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.¹⁻³ Tryptophan is also a precursor to the neurotransmitter melatonin, which is heavily involved in regulating the body's sleep cycle.¹⁻³

The Tryptophan Assay Kit 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 $\lambda_{\text{Ex}} = 530$ nm/ $\lambda_{\text{Em}} = 585$ nm is directly proportional to tryptophan concentration in the sample.

The linear detection range of the kit is 10 to 400 μM Tryptophan. The kit is suitable for tryptophan concentration determination in serum.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Enzyme Mix 12 mL
Catalogue Number MAK523A
- TRP Enzyme 120 μL
Catalogue Number MAK523B
- Dye Reagent 120 μL
Catalogue Number MAK523C
- Tryptophan Standard (5 mM) 100 μL
Catalogue Number MAK523D

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescent multiwell plate reader.
- Black flat-bottom 96-well or 384-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials before prior to opening. Equilibrate all components to room temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Note: This procedure is written for 96-well plates.

Sample Preparation

Samples require an internal Standard and need three separate reactions:

Internal Standard - prepare 500 μL of 100 μM tryptophan standard by mixing 10 μL 5 mM Standard and 490 μL purified water.

Sample plus Standard - add 5 μL of the 100 μM tryptophan and 10 μL Sample.

Sample alone - add 10 μL Sample and 5 μL purified water.

Sample blank - add 10 μL Sample and 5 μL purified water.

Working Reagent Preparation

Prepare enough Working Reagent for all Samples plus Standards and Samples alone as per Table 1.

Table 1.

Working Reagent Preparation

Reagent	Volume (μL)
Enzyme Mix	105
Dye Reagent	1
TRP Enzyme	1

Blank Working Reagent Preparation

Prepare enough Blank Working Reagent for Sample blank wells as per Table 2.

Table 2.

Blank Working Reagent Preparation

Reagent	Volume (μL)
Enzyme Mix	105
Dye Reagent	1

1. Add 100 μL of Working Reagent to each Sample plus Standard and Sample alone well.
2. Add 100 μL Blank Working Reagent to each Sample blank well.
3. Tap plate to mix briefly and thoroughly.

Measurement

1. Incubate plate protected from light for 30 min at room temperature.
2. Read fluorescence (F) at $\lambda_{\text{Ex}} = 530 \text{ nm} / \lambda_{\text{Em}} = 585 \text{ nm}$.

Results

Calculations

The sample tryptophan concentration is computed as follows:

Tryptophan (μM) =

$$\frac{F_{\text{Sample}} - F_{\text{Blank}}}{F_{\text{Standard}} - F_{\text{Sample}}} \times \frac{[\text{Standard}]}{2} \times \text{DF}$$
$$= \frac{F_{\text{Sample}} - F_{\text{Blank}}}{F_{\text{Standard}} - F_{\text{Sample}}} \times 50 \times \text{DF}$$

Where:

F_{SAMPLE} = Fluorescence readings of the Sample.

F_{STANDARD} = Fluorescence readings of the Sample plus standard.

F_{BLANK} = Fluorescence readings of the Sample blank.

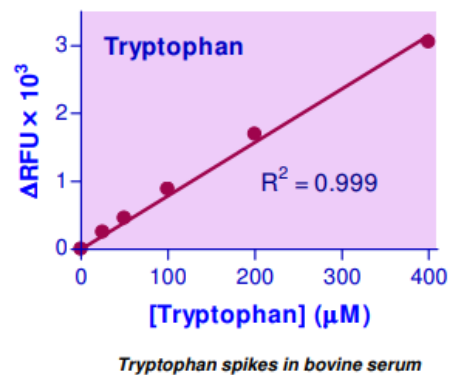
DF = Sample dilution factor.

Note: 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 dH_2O and repeat assay. Multiply result by the dilution factor DF.

Conversions: 1 μM tryptophan equals 0.204 mg/L, 0.0020% or 0.204 ppm

Figure 1.

Typical Tryptophan Activity Curve



References

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

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