



Tryptophan

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1245

(Version 2.2C)

Detection and Quantification of Tryptophan Content in Serum,
Plasma, Tissue extracts, Cell lysate, Cell culture media and Other
biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	4
IV. REAGENT PREPARATION.....	5
V. SAMPLE PREPARATION.....	6
VI. ASSAY PROCEDURE.....	7
VII. CALCULATION.....	8
VIII. TYPICAL DATA.....	9

I. INTRODUCTION

Tryptophan is an α -amino acid that is used in the biosynthesis of proteins.

Tryptophan contains an α -amino group, an α -carboxylic acid group, and a side chain indole, making it a non-polar aromatic amino acid. It is essential in humans, meaning the body cannot synthesize it; it must be obtained from the diet. Tryptophan is also a precursor to the neurotransmitter serotonin, the hormone melatonin and vitamin B3. Tryptophan is important to the functions of many organs in the body. When you consume tryptophan, your body absorbs it and changes it to eventually become a hormone called serotonin.

Tryptophan Colorimetric Microplate Assay Kit provides a sensitive and specific assay for determining Tryptophan in a variety of samples. The kit is based on Tryptophanase catalyzed decomposition of Tryptophan. The intensity of the product color, measured at 570 nm, is proportional to the Tryptophan concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	4 ml x 1	4 °C
Diluent	2 ml x 1	4 °C
Coenzyme	Powder x 1	4 °C, keep in dark
Enzyme	Powder x 1	-20 °C
Dye Reagent Diluent	15 ml x 1	4 °C
Dye Reagent	Powder	4 °C, keep in dark
Standard	Powder x 1	4 °C, keep in dark
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to dissolve to generate 10 mmol/L of standard top solution, store at 4 °C for 2-3 days or -20°C for 1-2 months after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Keep in dark and store at 4 °C for 1 week or -20 °C for 1-2 months.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Store at -20 °C for 2-3 weeks.

Dye Reagent: Briefly centrifuge prior to opening. Add 15 ml Dye Reagent Diluent and heat to 40°C for 5 minutes to dissolve before use. Keep in dark and store at 4 °C for 1 month or -20°C for 3-4 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detection directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	40 µl	40 µl	40 µl
Coenzyme	10 µl	10 µl	10 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, put it into the convection oven, 37 °C for 30 minutes,			
Dye Reagent	150 µl	150 µl	150 µl
Mix thoroughly, record absorbance measured at 570 nm immediately.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n \text{ (mmol/L)}$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times C_{\text{Protein}} \times V_{\text{Sample}}} \text{ (}\mu\text{mol/mg)}$$

2.2 According to the quantity of cells or bacteria

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}})} \text{ (}\mu\text{mol}/10^4)$$

2.3 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} \text{ (}\mu\text{mol/g)}$$

2.4 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} \times n \text{ (}\mu\text{mol/ml)}$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard}: the standard concentration, mmol/L = $\mu\text{mol/ml}$

V_{Standard}: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

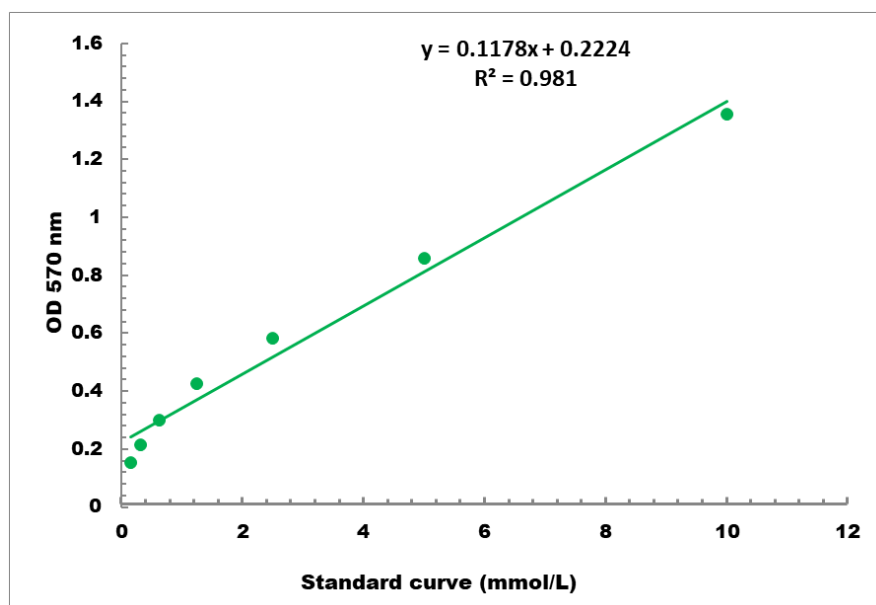
C_{Protein}: the sample protein concentration, mg/ml

W: the weight of sample, g

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 10 mmol/L