TryptophanMicroplate Assay Kit

User Manual

Catalog # CAK1245

(Version 1.2A)

Detection and Quantification ofTryptophanContentin Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

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 Microplate Assay Kit provides a convenient tool for sensitive detection of Tryptophan in a variety of samples. The intensity of the product color, measured at 600 nm, is proportional to the Tryptophan concentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 2	4 °C
Assay Buffer II	30 ml x 2	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	18 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Dye Reagent: add 18 ml Dye Reagent Diluent to dissolve before use.

Standard: add 1 ml distilled waterto dissolve before use, the concentration will be 10mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 600 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5mlAssay Buffer I for 5 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); then add 0.5mlAssay Buffer II,centrifuged at 10,000g for 10minutes, take the supernatant into a new centrifuge tube for detection.

2.For tissue samples

Weigh0.1 g tissue, homogenize with 0.5mlAssay Buffer I, incubate at 40°Cwater bath for 30 minutes;then add 0.5mlAssay Buffer II, centrifuged at 10,000g for 10minutes, take the supernatant into a new centrifuge tube for detection.

3. For serum or plasma samples

Detect directly, or dilute with distilled water.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	20µl	--	--
Standard	--	20µl	--
Distilled water	--	--	20µl
Dye Reagent	180 µl	180 µl	180 µl
Mix, cover the plate adhesive strip, put the plate into the convection oven,90°C for 10minutes. When cold, record absorbance measured at 600 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

Tryptophan (μmol/g) $=(C_{Standard}\times V_{Standard})\times(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$

$= 10\times(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

2.According to the quantity of cells or bacteria

Tryptophan (μmol/10^4) $=(C_{Standard}\times V_{Standard})\times(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$

$= 10\times(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

3. According to the volume of serum or plasma

Tryptophan (μmol/ml) $=(C_{Standard}\times V_{Standard})\times(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$

$= 10\times(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

$C_{Standard}$: the concentration of standard, 10mmol/L = 10μmol/ml;

W: the weight of sample, g;

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

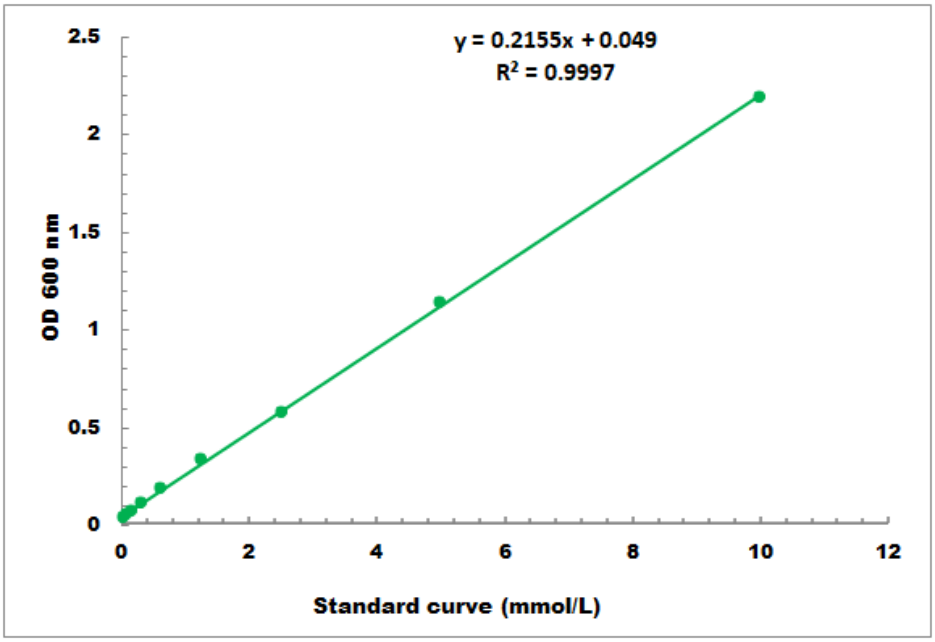
$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay Buffer I and Assay Buffer II, 1 ml.

VII. TYPICAL DATA

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



Detection Range: 0.05 mmol/L - 10 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES