Product Manual

Tryptophan Assay Kit

Catalog Number

MET-5177 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

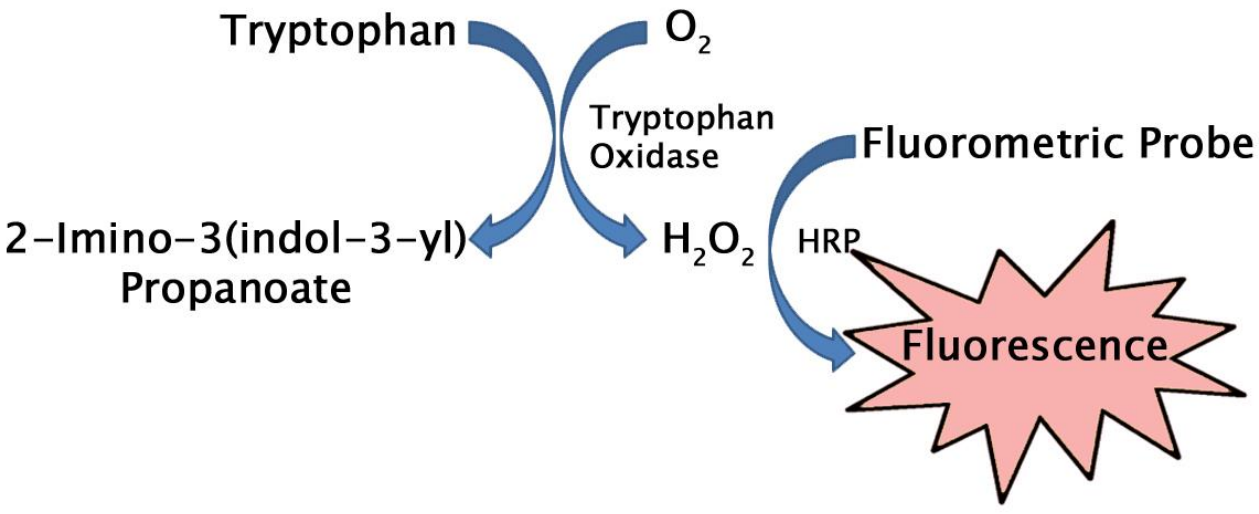
Tryptophan is an essential amino acid (obtained from the diet) that is used in protein biosynthesis. Tryptophan is a polar molecule since it contains an α-amino group, an α-carboxylic acid group, and a side chain indole. Tryptophan is a precursor for the synthesis of the neurotransmitter serotonin, the hormone melatonin, and vitamin B3. Serum albumin (such as BSA) binds tryptophan in a 1:1 ratio. Tryptophan is sold in the United States and the United Kingdom as a dietary supplement to treat depression, anxiety, and insomnia. Tryptophan is also sold under prescription in some European countries to treat major depression.

Cell Biolabs' Tryptophan Assay Kit is a simple assay for measuring tryptophan levels in biological samples. Total tryptophan levels may be quantified in serum or plasma following a special pretreatment protocol (see Preparation of Samples section). The kit has a detection sensitivity limit of 1.56 µM tryptophan. Each kit provides sufficient reagents to perform up to 100 assays*, including standard curve and unknown samples.

Note: Each sample replicate requires 2 assays, one treated with Tryptophan Oxidase (+TO) and one without (-TO). Tryptophan level is calculated from the difference in RFU readings from the 2 wells.

Assay Principle

The Tryptophan Assay Kit is a sensitive quantitative fluorometric assay for tryptophan. Tryptophan Oxidase converts tryptophan to 2-imino-3(indol-3-yl) propanoate and hydrogen peroxide (H_2O_2). The H_2O_2 is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples and standards are incubated for 60 minutes and then read with a standard 96-well fluorometric plate reader. Samples are compared to a known concentration of tryptophan standard within the 96-well microtiter plate format.



Related Products

1. MET-5054: L-Amino Acid Assay Kit
2. MET-5056: Branched Chain Amino Acid Assay Kit
3. MET-5071: Taurine Assay Kit
4. MET-5073: Tyrosine Assay Kit
5. MET-5129: Lysine Assay Kit (Fluorometric)

Kit Components

1. L-Tryptophan Standard (Part No. 51771C): One 100 µL vial at 10 mM.
2. 10X Assay Buffer (Part No. 51772A): One 30 mL bottle
3. Tryptophan Oxidase (Part No. 51773D): One 500 µL vial.
4. Fluorometric Probe (Part No. 50231C): One 50 µL tube in DMSO.
5. HRP (Part No. 234402): One 100 µL tube of a 100 U/mL solution in glycerol.

Materials Not Supplied

1. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range
2. 6.12 M Trichloroacetic Acid (CAS # 76-03-9)
3. 6 M Sodium Bicarbonate (CAS # 144-55-8)
4. 10 kD cutoff Centrifugal Filter Device

Storage

Upon receipt, store the Tryptophan Oxidase at -80°C. Store all other components at -20ºC. Avoid multiple freeze/thaw cycles. The Fluorometric Probe is light sensitive and must be stored accordingly.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.

- Reaction Mix: Prepare mixture according to the table below. Scale appropriately as needed and prepare only enough for immediate use.

Component	Reaction Mix (20 assays)
Fluorometric Probe	10 µL
HRP	2 µL
Tryptophan Oxidase	100 µL
1X Assay Buffer	888 µL
Total	**1000 µL**

- Negative Control Mix: Prepare mixture according to the table below. Scale appropriately as needed and prepare only enough for immediate use.

Component	Negative Control Mix (20 assays)
Fluorometric Probe	10 µL
HRP	2 µL
1X Assay Buffer	988 µL
Total	**1000 µL**

Preparation of Samples

- Cell culture supernatants: To remove insoluble particles, centrifuge at 10,000 x g for 10 min at 4°C. Collect the supernatant and filter the solution with a 10 kDa spin filter to deproteinate the sample. Collect flow through. The flow through can be assayed directly or diluted as necessary into 1X Assay Buffer. Prepare the Tryptophan standard curve in the same non-conditioned deproteinated media.

  Note: Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).

- Cell lysates: Resuspend cells in 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge 10,000 x g for 10 minutes at 4°C to remove debris. Collect the supernatant and filter the solution with a 10kD cutoff Centrifugal Filter Device to deproteinate the sample. Collect flow through. The flow through may be assayed undiluted or diluted as necessary into 1X Assay Buffer.

- Tissue lysates: Sonicate or homogenize tissue sample in 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect the supernatant and filter the solution with a 10kD cutoff

Centrifugal Filter Device to deproteinate the sample. Collect flow through. The flow through may be assayed directly or diluted as necessary into 1X Assay Buffer.

- Serum or Plasma

    - Free Tryptophan: Deproteinate human serum by loading 500 µL into a 10kD cutoff Centrifugal Filter Device. Spin 14000 xg for 10-30 minutes and collect the flow through. Store solution at -20°C until ready to test (dilute into 1X Assay Buffer as necessary).
    - Total Tryptophan (Tryptophan needs to be peripherally extricated from serum albumin): Dilute 6.12 M trichloroacetic acid (TCA) 1:3 to 2.04 M in distilled water. Add 100 µL of 2.04 M TCA to 400 µL of serum or plasma in a microcentrifuge tube and mix by pipetting up and down. Centrifuge the sample at 13000xg for 5 minutes at 4°C. Transfer 380 µL of supernatant to a fresh microcentrifuge tube. Neutralize by slowly adding 125 µL of 6 M sodium bicarbonate ($NaHCO_3$) ***(Important Note: add $NaHCO_3$ DROPWISE to avoid bubbling)***. Mix the solution gently by pipetting up and down. Store solution at -20°C until ready to test (dilute into 1X Assay Buffer as necessary).

- Urine: To remove insoluble particles, centrifuge at 10000 x g for 10 min at 4°C. Collect the supernatant and filter the solution with a 10kD cutoff Centrifugal Filter Device to deproteinate the sample. Collect flow through. The flow through can be assayed directly or diluted as necessary into 1X Assay Buffer.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since Resorufin is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Tryptophan standards before use by diluting in distilled water according to Table 1 below.

Standard Tubes	10 mM L-Tryptophan Solution (µL)	Distilled Water (µL)	L-Tryptophan (µM)
1	5	495	100
2	250 of Tube #1	250	50
3	250 of Tube #2	250	25
4	250 of Tube #3	250	12.5
5	250 of Tube #4	250	6.25
6	250 of Tube #5	250	3.13
7	250 of Tube #6	250	1.56
8	0	250	0

Table 1. Preparation of L-Tryptophan Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each unknown sample replicate requires two paired wells, one to be treated with Tryptophan Oxidase (+TO) and one without the enzyme (-TO) to measure endogenous background.
2. Add 50 µL of each Tryptophan Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix *(+TO)* to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
4. Add 50 µL of Negative Control Mix *(-TO)* to the other half of the paired sample wells.
5. Mix the well contents thoroughly and incubate for 60 minutes at room temperature protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.

6. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.

Calculation of Results

1. Determine the average relative fluorescence unit (RFU) values for each sample, control, and standard.
3. Subtract the average zero standard value from itself and all standard values.
4. Graph the standard curve (see Figure 1).
5. Subtract the sample well values without Tryptophan Oxidase *(-TO)* from the sample well values containing Tryptophan Oxidase (+*TO*) to obtain the difference. The fluorescence difference is due to the Tryptophan Oxidase activity:

$$\text{Net RFU} = \text{RFU}_{(+TO)} - \text{RFU}_{(-TO)}$$

6. Compare the Net RFU of each sample to the standard curve to determine and extrapolate the quantity of Tryptophan present in the sample. Only use values within the range of the standard curve.
7. To calculate the final tryptophan concentration in the sample, take into account any prior dilutions performed (for example in the total tryptophan plasma/serum protocol, sample is diluted 6.6-fold)

Example of Results

The following figures demonstrate typical Tryptophan Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

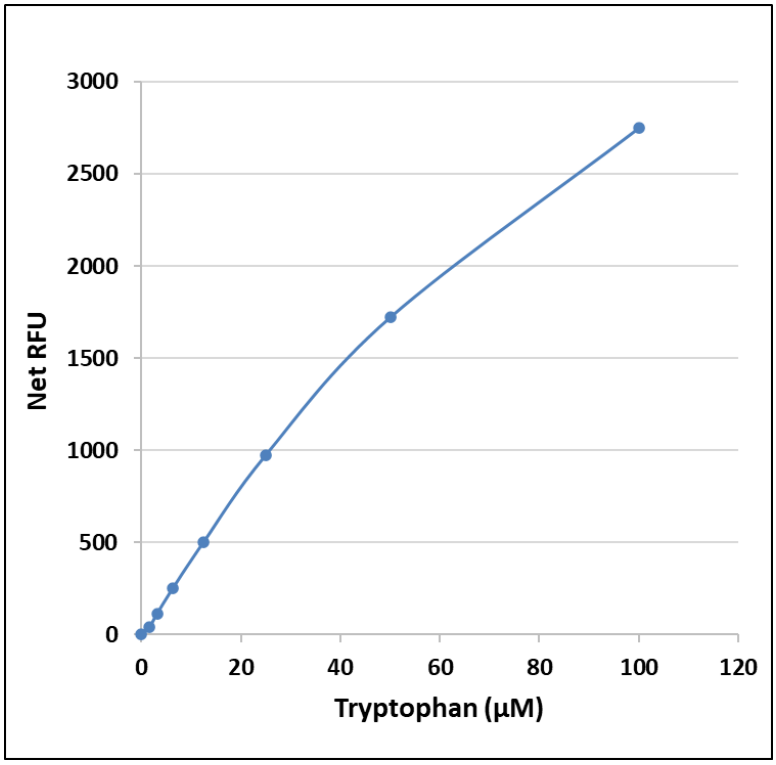


Figure 1. Tryptophan Standard Curve.

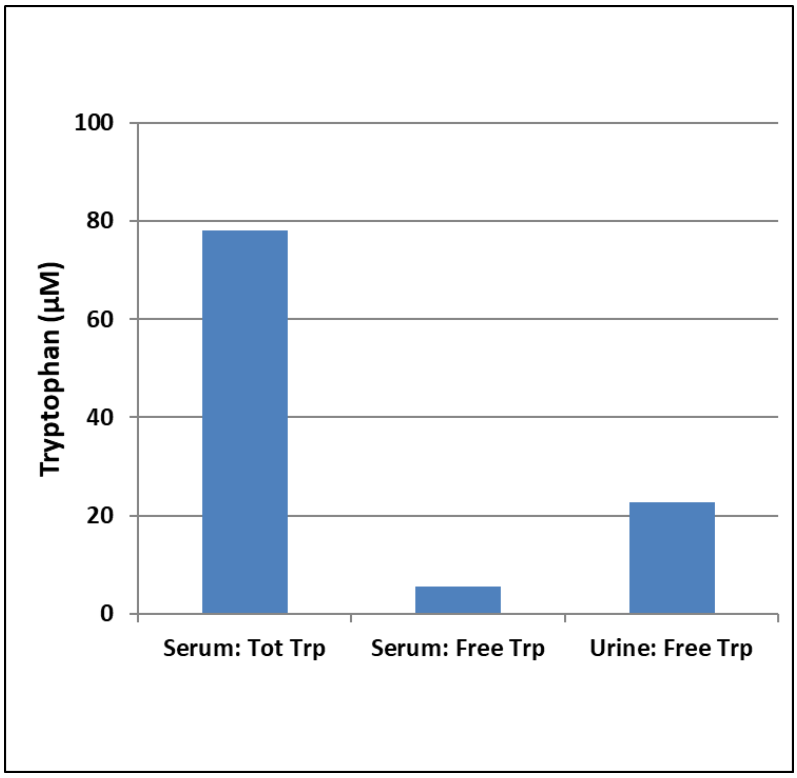


Figure 2. Detection of total or free tryptophan in human serum or urine. Human serum or urine was deproteinated according to the preparation of samples section above.

References

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Warranty

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