Tryptophan (Trp) Assay Kit

Catalog No.: abx298937

Size: 100 Assays

Storage: Store all components at 4°C.

Application: For quantitative detection of Tryptophan concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.

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

Introduction: Tryptophan (Trp) is an alpha amino acid, and is a key constituent of proteins. Humans are unable to synthesize Trp, primarily obtaining it through diet. Tryptophan is also a precursor for several signaling compounds, including the neurotransmitter serotonin, and the hormone melatonin. As a result, Tryptophan is a target of interest in neuroscience, medicinal science and physiology.

Abbexa's Tryptophan (Trp) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Trp concentrations. The dye reagents react with Trp to create an absorption maximum at 600 nm. The intensity of the color is proportional to the concentration of Trp, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer 1: 2 × 30 ml
3. Assay Buffer 2: 2 × 30 ml
4. Dye Reagent: 1 vial
5. Dye Reagent Diluent: 18 ml
6. Standard: 1 vial
7. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (600 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar
9. Water bath/convection oven

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent Solution**
  Add 18 ml of Dye Reagent Diluent into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. If any precipitates are observed, warm the vial using a water bath until the precipitates have dissolved.

- **Standard Solution**
  Add 1 ml of distilled water into the Standard vial and mix thoroughly to prepare the Standard Solution (concentration 10 mmol/L). Ensure that the Standard has completely dissolved.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 0.5 ml of Assay Buffer 1 for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Add 0.5 ml of Assay Buffer 2, and centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 0.5 ml of Assay Buffer 1 on ice. Incubate at 40°C in a water bath for 30 minutes. Add 0.5 ml of Assay Buffer 2, then centrifuge at 10,000 × g for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

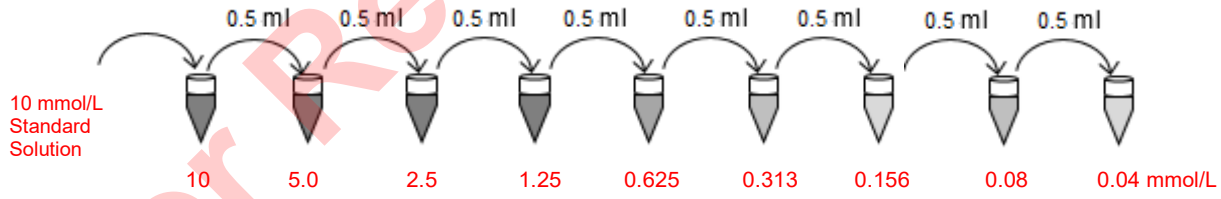
- **Liquid (serum, plasma) samples**
  Liquid samples can be used directly. If dilution is required, dilute with distilled water.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 9 tubes with 10 mmol/L, 5.0 mmol/L, 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.313 mmol/L, 0.156 mmol/L, 0.08 mmol/L, and 0.04 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 10 mmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 20 µl of sample to the sample wells.
4. Add 20 µl of prepared standards to the standard wells.
5. Add 20 µl of distilled water to the blank wells.
6. Add 180 µl of Dye Reagent Solution to all wells.
7. Cover the plate and incubate at 90°C in a convection oven for 10 minutes.
8. Allow to cool to room temperature, then read and record absorbance at 600 nm.

C. Calculations

Plot the standard curve, using the OD of the standard dilutions on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula y=ax+b. Based on this curve, the concentration of Trp in each sample well can be derived with the formula:

Tryptophan concentration per g of sample:

$$\textbf{Tryptophan}\ (\mu\textbf{mol/g}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{10}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Tryptophan concentration per 10^4 cells or bacteria:

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

Tryptophan concentration per ml serum or plasma:

$$\textbf{Tryptophan}\ (\mu\textbf{mol/ml}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \mathbf{10} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

$\mathbf{C_{Standard}}$	Concentration of highest standard (10 mmol/L= 10 µmol/ml)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{N}$	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Total volume of Assay Buffer 1 and Assay Buffer 2 (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.02 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.02 ml)