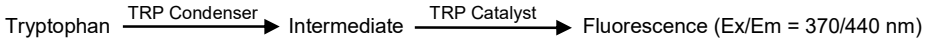
Tryptophan Assay Kit (Fluorometric) (BN00787)

(Catalog # BN00787; 100 assays; Store at 4°C)

I. Introduction:

Tryptophan (TRP) is one of the eight essential amino acids. In general, TRP is the least abundant amino acid in humans. Chemically, TRP side chain (indole) confers its unique fluorometric properties. External sources of tryptophan include chicken, tuna, bananas, cheese, chocolate etc. Tryptophan is mainly used for protein synthesis. Additionally, TRP serves as a building block for several metabolites including kynurenine, serotonin, tryptamine, melatonin, niacin, and NAD/NAPD. TRP is the only amino acid that can be found in blood in two forms: bound (BTRP) and free (FTRP) Tryptophan. Changes in tryptophan concentrations are directly related to a number of physiological and behavioral processes including: sleep, memory, depression, motion sickness, bipolar disorders, and schizophrenia. Assay Genie's Tryptophan Assay Kit provides a simple, sensitive, and high-throughput adaptable assay that detects tryptophan concentration in biological fluids, including free and bound tryptophan in serum. The principle is based on the non-enzymatic reaction to generate a fluorophore (Ex/Em = 370/440 nm) using tryptophan as a building block. The reaction is specific and other amino acids do not interfere with the assay. The assay can detect as little as 2.5 µM of TRP in a variety of biological samples.



II. Application:

- Estimation of Tryptophan in biological samples

III. Sample Type:

- Biological fluids such as serum, urine etc.

IV. Kit Contents:

Components	BN00787	Cap Code	Part Number
TRP Deproteinization Reagent	3 ml	NM	BN00787-1
TRP Neutralization Solution	4 ml	NM	BN00787-2
TRP Condenser	2 ml	Red	BN00787-3
TRP Catalyst	2 ml	Blue	BN00787-4
TRP Standard (25 mM)	0.1 ml	Yellow	BN00787-5

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- PCR tubes
- 10 kDa Spin Column
- Multi-well spectrophotometer
- PCR thermal cycler or heat block

VI. Storage Conditions and Reagent Preparation:

Store kit at 4°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **TRP Deproteinization Reagent, TRP Neutralization Solution, TRP Condenser, and TRP Catalyst**: Bring to room temperature (RT) before use. Store at 4°C.

VII. Tryptophan Assay Protocol:

1. **Sample Preparation:** Dilute urine sample (recommended Dilution Factor: 10) with water. To measure total Tryptophan (TTRP) level in serum, first dilute TRP Deproteinizing reagent three times with dH_2O (i.e. 1 part Reagent + 2 parts Water). Add 100 µl of diluted TRP Deproteinizing Reagent to 400 µl of serum, vortex samples to mix well, and keep on ice. Centrifuge at 13,000 x g, 4°C for 5 min. Transfer the supernatant (~ 380 µl) to another centrifuge tube. Add 20 µl of TRP Neutralization Solution to neutralize the sample. Keep the sample on ice for 5 min., and vent the sample tube, as there may be some released CO_2. Add 1-30 µl of urine or serum sample into PCR tube. Adjust the volume to 110 µl per tube with ddH_2O

   Notes:

   a. Deproteinized samples may be stored at -70°C for up to a month without neutralization.

   b. Tryptophan concentrations can vary over a wide range, depending on the sample. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

   c. For samples having high background, prepare parallel sample well(s) as sample background control(s). Adjust the volume to 110 µl with ddH_2O.

   d. Free Tryptophan: To measure free Tryptophan in serum (FTRP), deproteinize samples using a 10 kDa Spin Colum (not provided). Add sample to the spin column and centrifuge (10000 x g, 10 min., 4°C). Collect the filtrate. Add 1-30 µl of filtrate into the PCR tube. Adjust the volume to 110 µl per tube with ddH_2O. Deproteinization using Spin Columns will only measure FTRP. It does not measure total Tryptophan in samples.

2. **Standard Curve Preparation:** Dilute TRP Standard to 0.25 mM by adding 10 µl of 25 mM Standard to 990 µl of ddH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 0.25 mM TRP Standard into a series of PCR tubes to generate 0, 0.5, 1, 1.5, 2, and 2.5 nmol TRP/tube. Adjust the volume to 110 µl/tube with ddH_2O.

3. **Reaction Mix:** Add 20 µl of TRP Condenser to the PCR tubes containing Standards, samples and sample background control. Mix well and incubate at room temperature for 2 min. Subsequently add 20 µl of TRP catalyst to Standard, and sample PCR tubes. Mix well. Incubate tubes at 105°C for 60 min. in a PCR thermal cycler or heat block. Keep PCR tubes on ice for 10 min. Gently tap the tubes to bring the content at the bottom of the tube. Transfer 130 µl into a 96-well white microplate.

   Notes:

   a. TRP Condenser and TRP Catalyst should be added sequentially.

   b. Don't add TRP catalyst to the sample background control well(s).

4. **Measurement:** Measure fluorescence (Ex/Em = 370/440 nm) as end point.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Tryptophan Standard Curve. Apply sample's corrected RFU to Standard Curve to get B nmol of Tryptophan.

   Note: If sample background control reading is significant, then subtract sample background control reading from sample reading.

Sample Tryptophan Concentration (C) = B/V X D nmol/µl or mM

Where: **B** is amount of Tryptophan in the sample well from Standard Curve (nmol)
V is sample volume added into the reaction well (µl)
D is sample dilution factor (1.3 in case of total Tryptophan in serum)

Tryptophan molecular weight: 204.23 g/mol
1 mM ≡ 1000 µM ≡ 204 µg/ml

Note: The above equation can be used to calculate both the total and free tryptophan in serum.

Use the following equation to determine the bound Tryptophan in serum

Serum Total Tryptophan = Serum Free Tryptophan + Serum Bound Tryptophan

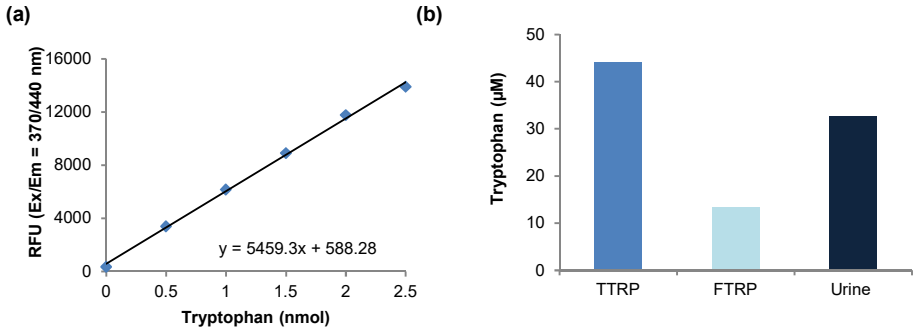


Figure: (a) Tryptophan Standard Curve. **(b)** Estimation of Tryptophan in human serum and urine. To measure total Tryptophan and free Tryptophan levels in serum, samples were deproteinized using the kit protocol. TTRP: Total Tryptophan in serum, FTRP: Free Tryptophan in serum. Serum total Tryptophan (10 µl, undiluted), serum free Tryptophan (30 µl, undiluted), and urine (25 µl, 10-fold diluted) samples were assayed following the kit protocol. Estimated concentrations of Tryptophan (in µM): total Tryptophan in serum: 44.2; free Tryptophan in serum: 13.7; bound Tryptophan in serum: 30.5; Total Tryptophan in urine: 32.6.

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