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Product Information

Tryptophan Assay Kit

Catalog Number **MAK254**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Tryptophan (TRP) is one of the eight essential amino acids in humans, and, in general, is the least abundant amino acid. The indole side chain of tryptophan is responsible for its intrinsic fluorescence properties. Tryptophan is mainly used for protein synthesis. In addition, TRP serves as a building block for several metabolites including kynurenine, serotonin, tryptamine, melatonin, niacin, and NAD/NAPD. It 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. External sources of tryptophan include chicken, tuna, bananas, cheese, and chocolate.

The Tryptophan Assay Kit measures the tryptophan concentration in biological fluids, including free and bound tryptophan in serum. The assay is based on a non-enzymatic reaction in which tryptophan is used as a building block to generate a fluorometric product (λ_{ex} = 370 nm/λ_{em} = 440 nm). The reaction is specific and other amino acids do not interfere with the assay. This high-throughput adaptable assay kit is simple and sensitive enough to detect to 2.5 μM of tryptophan in a variety of biological samples.

This kit is suitable for use with serum, plasma, urine and other biological fluids

Components

The kit is sufficient for 100 assays in 96 well plates.

Component	Amount
TRP Deproteinization Reagent Catalog Number MAK254A	3 mL
TRP Neutralization Solution Catalog Number MAK254B	4 mL
TRP Condenser Catalog Number MAK254C	2 mL
TRP Catalyst Catalog Number MAK254D	2 mL
TRP Standard (25 mM) Catalog Number MAK254E	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate. It is recommended to use white plates for fluorescence assays.
- Fluorescence multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter
- PCR tubes
- PCR thermal cycler or heat block

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

TRP Deproteinization Reagent, TRP Neutralization Solution, TRP Condenser, and TRP Catalyst – Allow to come to room temperature before use. Store at 2–8 °C.

Storage/Stability

The kit is shipped on wet ice. Storage at 2–8 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of standards and samples.

TRP Standards for Fluorometric Detection

Dilute 10 μL of the 25 mM TRP Standard with 990 μL of water to prepare a 0.25 mM standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 0.25 mM TRP standard solution into a series of PCR tubes, generating 0 (blank), 0.5, 1, 1.5, 2, and 2.5 nmol/well standards. Add water to each well to bring the volume to 110 μL.

Sample Preparation

Dilute urine samples 1:10 with water.

1. Total Tryptophan (TTRP) level in serum

To measure the TTRP level in serum, dilute the TRP Deproteinization Reagent 1:3 with water. Add 100 μL of diluted TRP Deproteinization Reagent to 400 μL of serum and vortex samples to mix well. Keep samples on ice. Centrifuge samples at 13,000 × *g*, for 5 minutes at 2–8 °C. Collect the supernatant (~380 μL) and transfer it to a new centrifuge tube. Add 20 μL of TRP Neutralization Solution to neutralize the sample. Keep the sample on ice for 5 minutes, and vent the sample tube, as there may be some CO_2 build-up.

Add 1–30 μL of samples into duplicate PCR tubes. Bring samples to a final volume of 110 μL per tube with water.

Notes: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve.

For samples having high background, prepare parallel sample wells as sample background controls, Adjust the final volume to 110 μL with water.

2. Free Tryptophan (FTRP) level in serum

To measure the FTRP level, deproteinize samples using a 10 kDa MWCO spin filter prior to addition to the reaction. Deproteinization will only measure FTRP. It does not measure total Tryptophan in samples.

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

Assay Reaction

1. Add 20 μL of TRP Condenser to each sample, standard, and background control PCR tube. Mix well and incubate at room temperature for 2 minutes.

2. Add 20 μl of TRP Catalyst to each sample and standard PCR tube. Mix well. Incubate at 105 °C for 60 minutes in a PCR thermal cycler or heat block. Remove samples and incubate on ice for 10 minutes. Allow the contents to settle at the bottom of the tube, then transfer 130 μL into a 96 well plate.

Notes: TRP Condenser and TRP Catalyst should be added sequentially.

Do not add TRP catalyst to the sample background control well(s).

3. Measure fluorescence intensity (λ_{ex} = 370/ λ_{em} = 440 nm).

Results

The background is the value obtained for the 0 (assay blank) TRP Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Subtract the Sample Background Control value from the sample readings. Note: A new standard curve must be set up each time the assay is run.

Use the values obtained from the appropriate TRP Standards to plot a standard curve. The amount of TRP present in the sample may be determined from the standard curve.

Concentration of Tryptophan

Total and Free Tryptophan in serum:

$$C = \frac{S_a}{S_v \times S_d}$$

S_a = Amount of TRP in the unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
S_d = Sample dilution factor (i.e. 1:3 in total TRP in serum)
C = Concentration of TRP in sample

Tryptophan molecular weight: 204.23 g/mole

Bound Tryptophan in serum:

$$Serum\ Total\ Tryptophan = Serum\ Free\ Tryptophan + Serum\ Bound\ Tryptophan$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use white plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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