Product Manual

Tyrosine Assay Kit

Catalog Number

MET-5073 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Tyrosine (also known as 4-hydroxyphenylalanine) is one of the 20 amino acids that serve as building blocks to form peptides and proteins. As a part of proteins, tyrosine plays a role in signal transduction since phosphate groups are transferred to the hydroxyl group of the phenolic side chain of tyrosine by receptor tyrosine kinases, resulting in a change in activity of the target phosphorylated protein. In addition, a tyrosine side chain contributes to the process of photosynthesis. In photosystem II of chloroplasts, tyrosine residue 161 of P680 acts as an electron donor to reduce oxidized chlorophyll. In plants and lower microorganisms, tyrosine is synthesized from the precursor prephenate (the latter also an intermediate to synthesis of shikimate). Prephenate is decarboxylated to create p-hydroxyphenylpyruvate, which is then transaminated to yield tyrosine and α-ketoglutarate. In animals tyrosine is created from dietary sources of the essential amino acid phenylalanine. Phenylalanine is converted to tyrosine by the enzyme phenylalanine hydroxylase.

Tyrosine is a neurotransmitter precursor and increases the levels of plasma neurotransmitters such as norepinephrine and dopamine. A number of studies have found tyrosine to reduce stress under conditions of sleep deprivation, prolonged work, cold, or fatigue, and includes reductions in stress hormone levels, and stress-induced weight loss. In addition, improvements in cognitive and physical performance have been observed in humans.

Cell Biolabs' Tyrosine Assay Kit is a simple colorimetric assay that measures the total amount of free tyrosine present in foods or biological samples in a 96-well microtiter plate format. The Tyrosine Assay Kit can also detect L-DOPA, γ-L-glutaminyl-3,4-hydroxybenzene (GHB), and γ-glutaminyl-3,4-dihydroxybenzene (GDHB). Tyrosine is enzymatically oxidized to a colorimetric intermediate which is then measured with a standard 96-well spectrophotometric plate reader. Samples are compared to a known concentration of tyrosine standard within the 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, tyrosine standards, and unknown samples. Sample tyrosine concentrations are determined by comparison with a known tyrosine standard. The kit has a detection sensitivity limit of 15.6 µM tyrosine.

Related Products

1. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
2. MET-5055: L-Amino Acid Assay Kit (Fluorometric)
3. MET-5056: Branched Chain Amino Acid Assay Kit
4. MET-5070: Glycine Assay Kit
5. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
6. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit
7. STA-670: Homocysteine ELISA Kit
8. STA-674: Glutamate Assay Kit
9. STA-675: Hydroxyproline Assay Kit
10. MET-5071: Taurine Assay Kit

Kit Components

1. Tyrosine Standard (Part No. 50731C): One 100 µL vial at 100 mM.
2. 10X Assay Buffer (Part No. 50732A): One 25 mL bottle.
3. 10X Enzyme (Part No. 50733D): One 500 µL vial.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Microcentrifuge tubes
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Standard 96-well black microtiter plate and/or cell culture microplate
7. Multichannel micropipette reservoir
8. Microplate reader capable of reading at 490 nm

Storage

Upon receipt, store the Tyrosine Standard and 10X Enzyme at -20ºC. Store the 10X Assay Buffer at room temperature.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer to 1X with deionized water. Stir to homogeneity. Store at room temperature.
- Reaction Mix: Prepare a Reaction Mix by diluting the 10X Enzyme 1:10 in 1X Assay Buffer. For example, add 100 µL to 900 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Urine: Deproteinate the sample by running it through a centrifugal filter unit (e.g. Amicon Ultra 0.5 mL 10K Cat. No. UFC501024) and collecting the flow through. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Serum or Plasma: Deproteinate the sample by running it through a centrifugal filter unit (e.g. Amicon Ultra 0.5 mL 10K Cat. No. UFC501024) and collecting the flow through. To remove

insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed undiluted or diluted as necessary in 1X Assay Buffer.

Note: 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.

Preparation of Standard Curve

Prepare fresh Tyrosine Standards before use by diluting in 1X Assay Buffer according to Table 2 below.

Standard Tubes	100 mM Tyrosine Solution (µL)	1X Assay Buffer (µL)	Tyrosine (µM)
1	5	495	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.3
7	250 of Tube #6	250	15.6
8	0	250	0

Table 2. Preparation of Tyrosine 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.
2. Add 50 µL of each Tyrosine Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 10 minutes at room temperature on an orbital shaker.
4. Read the plate at 490 nm using a microplate spectrophotometer.

Example of Results

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

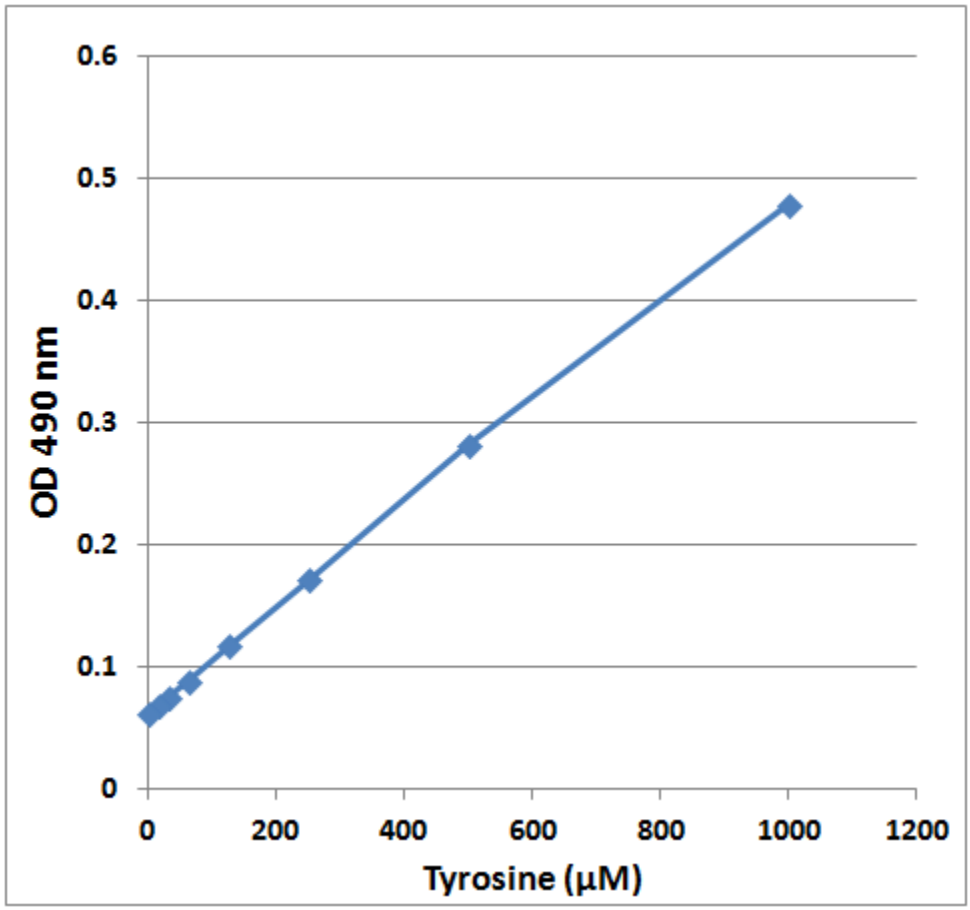

Figure 2: Tyrosine Standard Curve.

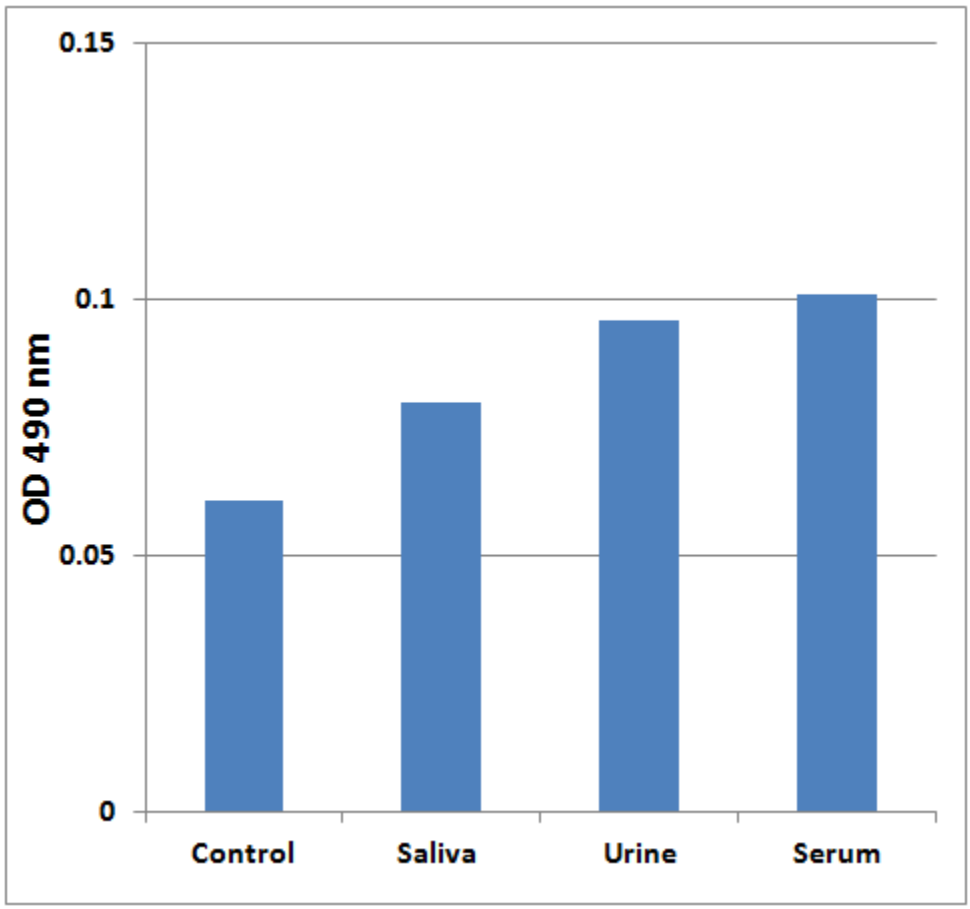

Figure 3: Tyrosine Detection in Normal Human Saliva, Urine or Serum. Tyrosine was detected in human samples. Samples were deproteinated and tested undiluted according to the Assay Protocol.

References

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Recent Product Citations

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2. Yang, Y. et al. (2019). A laser-engraved wearable sensor for sensitive detection of uric acid and tyrosine in sweat. *Nat Biotechnol*. doi: 10.1038/s41587-019-0321-x.

Warranty

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