



**User's Manual**

# **Tyrosine Colorimetric Assay Kit**



**DEIA100J**



**96T**



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

The 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.

### General Description

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  $\alpha$ -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.

### Principles of Testing

The 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,  $\gamma$ -L-glutaminy-3,4-hydroxybenzene (GHB), and  $\gamma$ -glutaminy-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  $\mu$ M tyrosine.

### Reagents And Materials Provided

1. Tyrosine Standard: One 100  $\mu$ L vial at 100 mM.
2. 10X Assay Buffer: One 25 mL bottle.
3. 10X Enzyme: One 500  $\mu$ L vial.

### Materials Required But Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Standard 96-well black microtiter plate and/or cell culture microplate

## Storage

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

## Specimen Collection And Preparation

- 1. Tissue lysates:** Sonicate or homogenize tissue sample in cold PBS or 1× Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1× Assay Buffer.
  - 2. Cell lysates:** Resuspend cells at  $1-2 \times 10^6$  cells/mL in PBS or 1× Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1× Assay Buffer.
  - 3. Urine:** Deproteinize the sample by running it through a centrifugal filter unit 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 1× Assay Buffer.
  - 4. Serum or Plasma:** Deproteinize the sample by running it through a centrifugal filter unit 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 1× 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.

## Reagent Preparation

- 1. 1× Assay Buffer:** Dilute the 10× Assay Buffer to 1× with deionized water. Stir to homogeneity. Store at room temperature.
  - 2. Reaction Mix:** Prepare a Reaction Mix by diluting the 10× Enzyme 1:10 in 1× Assay Buffer. For example, add 100 µL to 900 µL of 1× 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 4°C.
- Note:** Prepare only enough for immediate use by scaling the above example proportionally.
- 3. Preparation of Standard Curve:**
- Prepare fresh Tyrosine Standards before use by diluting in 1× Assay Buffer according to Table 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

## Assay Procedure

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.

## Typical Standard Curve

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.



