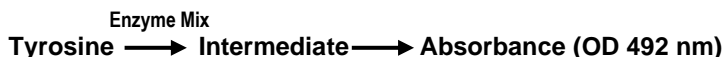


**DESCRIPTION**

Tyrosine (Tyr) is a nonessential amino acid. It makes from another amino acid called phenylalanine. Tyr is an essential component for the production of several important brain chemicals and hormones, including epinephrine, norepinephrine, dopamine, thyroid hormones, melanin, fumarate, and acetoacetate. The pathology of abnormal concentrations of Tyr is well-known in diseases including phenylketonuria, hypothyroidism, tyrosinemia, albinism, and alkaptonuria.

Tribioscience’s Tyrosine Colorimetric Assay kit is a simple, and sensitive approach that can detect normal and abnormal concentrations of Tyr in biological fluids. The assay is based on the enzymatic oxidation of Tyr producing a stable signal (OD 492 nm), which is directly proportional to the amount of Tyr. Sample preparation is minimal and does not require strenuous or complicated procedures. The assay can detect as low as 15 µM of Tyr in a variety of biological samples.

**ASSAY PRINCIPLE**



**APPLICATION**

- Measurement of Tyrosine in various biological samples.
- Analysis of Tyrosine in pathological conditions.

**KIT CONTENTS**

Component	100x Rxns
Tyrosine Enzyme Mix	300 µL
Tyrosine Standard (100 mM)	20 µL
Assay buffer	10 ml

Storage conditions: Store the kit at -20°C protected from light.  
Shelf life: 12 months.

**ASSAY PROCEDURES**

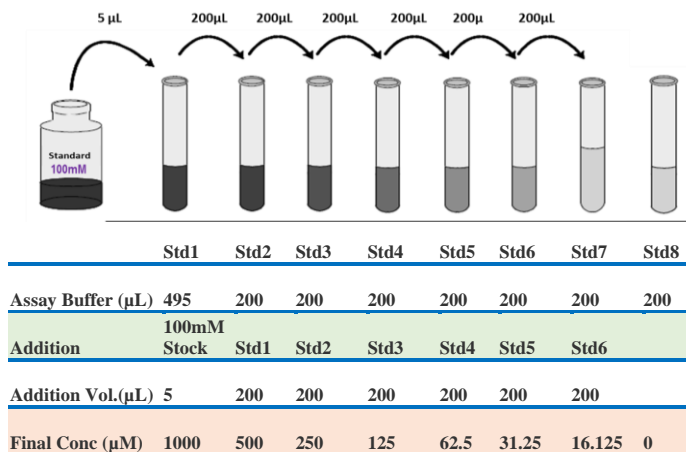
**1. Sample Preparation**

Collect cell culture supernatant, serum, plasma, urine, and other biological fluids. Deproteinizing the samples using 10 kDa Spin Column (Amicon Ultra 0.5 mL, Cat. No. UFC501024). Briefly, add sample to the spin column, centrifuge at 10,000 x g for 5 min. at 4°C. Collect the filtrate. Add 50 µl of filtrate into desired well(s) in 96-well plate.

**2. Standard Preparation as Fig.1:**

- 2.1 Label 8x 1.5mL tubes 1-8 for a standard curve as shown in the diagram below.
- 2.2 Add 495 µL of Assay Buffer to Std 1 and 200µL to Std 2-8.
- 2.3 Add 5 µL of 100 mM tyrosine Standard Stock solution to Std1, then carry out a 2x serial dilution for Std 2-7. Leave Std 8 as the 0 standard (the assay buffer alone). The standards concentrations are 1000, 500, 250, 125, 62.5, 31.25, 16.125 and 0 µM for Std 1-8.

**Fig.1 Diagram for Tyrosine Standard Preparation**



2.4 Load the samples: Pipet 50µL of standards, controls, and test samples into individual wells of a microplate in duplicate manner (Note: recommend running a pilot study to determine the optimal concentration of sample within the assay standard curve range).

2.5 Prepare Enzyme working solution for each well by mixing the reagent as following:

- 48 µL Assay solution
  - 2 µL Enzyme Mix solution
- Mix, then, add each well.

For 100 Assay: mix 5.28 mL Assay Buffer + 220uL Enzyme mix.

2.6 Begin the reactions. Add 50µL of above working solution to each microplate well containing the standards, controls, and samples.

2.7 Incubate the reactions. Incubate at room temperature for 60-minutes protected from light.

2.8 Measure absorbance using a microplate reader with excitation range of 492 nm.

2.9 Correct for background absorbance. For each point, subtract the value derived from the 0 control.

**Calculation**

Subtract the blank value (0µM Standard) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the Tyrosine concentration of the Sample using the equation obtained from the linear regression of the standard curve.

$$\text{Ty Concentration (uM)} = N \times (\text{Rsample} - \text{Rblank}) / \text{Slope (uM)}$$

Where: Rsample and Rblank are optical density readings of the sample and blank, respectively. N is the sample dilution factor.

**RELATED PRODUCTS:**

- Tyrosinase Activity Assay (TBS2072)
- ATP Activity Assay (TBS2010)
- Hydrogen Peroxidase Activity Assay Kit (TBS2067)
- HRP Fluorescence -System (TBS5026)
- Tryptase Activity Assay (TBS2101)
- β-Hexosaminidase Activity Assay (TBS2105)
- Cytochrome C Oxidase Activity Assay (TBS2115)
- Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
- Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
- Non-esterified Fatty Acid Assay (TBS2203)
- Glycerol Colorimetric / Fluorometric Assay (TBS2204)
- Protein Assay Kits (TBS2005)
- Cell Nuclear Extract kit (TBS6025)

**For research use only.**

