



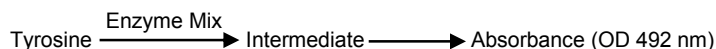
Tyrosine Colorimetric Assay Kit

rev. 11/13

(Catalog # K573-100; 100 assays; Store at -20°C)

I. Introduction:

Tyrosine (Tyr) is one of the four standard amino acids containing an aromatic group as a side chain. Its hydrophobicity is one of the main characteristics of this uncharged polar amino acid. In addition to being an essential amino acid Tyr is important in number of biological processes such as the synthesis of neurotransmitters, thyroid hormones, melanine, fumarate and acetoacetate. The pathology of abnormal concentrations of Tyr is well known in diseases including phenylketonuria, hypothyroidism, tyrosinemia, albinism, and alkaptonuria. BioVision's Tyrosine Assay kit is a simple, yet sensitive assay that is able to 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 50 µM of Tyr in a variety of biological samples.



II. Application:

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

III. Sample Type:

- Serum, plasma
- Urine or other body fluids

IV. Kit Contents:

Components	K573-100	Cap Code	Part Number
Tyr Assay Buffer	25 ml	WM	K573-100-1
Tyr Enzyme Mix (Lyophilized)	1 vial	Green	K573-100-2
Tyr Standard (Lyophilized)	1 vial	Yellow	K573-100-3

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer
- 10 kDa Spin Column (Cat. # 1997)

VI. Storage and Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **Tyr Assay Buffer:** Warm Assay Buffer to room temperature before use. Store at 4°C or -20°C.
- **Tyr Enzyme Mix:** Reconstitute with 220 µl Tyr Assay Buffer. Pipette gently to dissolve. Aliquot & store at -20°C. Keep on ice while in use. Stable for two months.
- **Tyr Standard:** Reconstitute with 100 µl ddH₂O to generate 100 mM solution. Store at 4°C. Stable for two months.

VIII. Tyrosine Assay Protocol:

1. Sample Preparation: Samples should be deproteinized using 10 kDa Spin Column (Cat. # 1997). Briefly, add sample to the spin column, centrifuge at 10,000 x g for 10 min. at 4°C. Collect the filtrate. Add 80-135 µl of filtrate into desired well(s) in 96-well plate. Adjust the volume to 150 µl/well with ddH₂O.

Notes:

- Tyrosine concentrations can vary over a wide range. Normal ranges in humans are 55-147 µM for serum, 10-290 µM for urine, and 34-112 µM in plasma. Abnormal tyrosine levels can exceed 1.5 mM in tyrosinemia samples. For unknown samples, we recommend doing pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.
- For samples having background, prepare parallel sample well(s) as background control(s).
- Endogenous compounds may interfere with the assay. To ensure accurate determination of Tyr in the test samples or for samples having low concentration of Tyr, we recommend spiking samples with a known amount of Tyr Standard (30 nmol).

2. Standard Curve Preparation: Dilute the Tyr Standard to 2.5 mM by adding 25 µl of 100 mM Tyr Standard to 975 µl of ddH₂O. Add 0, 2, 6, 12, 18, 24 & 30 µl of Tyr Standard into series of wells in a 96-well plate to generate 0, 5, 15, 30, 45, 60, & 75 nmol/well of Tyr Standard. Adjust the volume to 150 µl/well with ddH₂O. The diluted Standard can be stored at 4°C for subsequent assays.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	* Background Control Mix
Tyr Assay Buffer	48 µl	50 µl
Tyr Enzyme Mix	2 µl	---

Mix well. Add 50 µl of the Reaction Mix to each well containing Standards and samples. Mix.

* For samples having background, add 50 µl of Background Control Mix to sample background control well(s). Mix.



- 4. Measurement:** Incubate the plate at room temperature for 60 min., protected from light. Measure absorbance (OD 492 nm) in a microplate reader.
- 5. Calculation:** Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the Tyr Standard Curve. For unspiked samples, apply the corrected OD to the Tyr Standard Curve to get B nmol of Tyr in the sample well.

$$\text{Sample Tyr concentration (C)} = \text{B/V X D nmol/ml or } \mu\text{M}$$

Where: **B** is the amount of Tyr in the sample well from the standard curve (nmol)
V is the sample volume added into the reaction well (ml)
D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

$$\text{For spiked samples, Tyr amount in sample well (B)} = \left(\frac{(\text{OD}_{\text{sample (corrected)}})}{(\text{OD}_{\text{sample + Tyr Std (corrected)}}) - (\text{OD}_{\text{sample (corrected)}})} \right) * \text{Tyr Spike (nmol)}$$

Tyrosine molecular weight: 181.2 g/mol

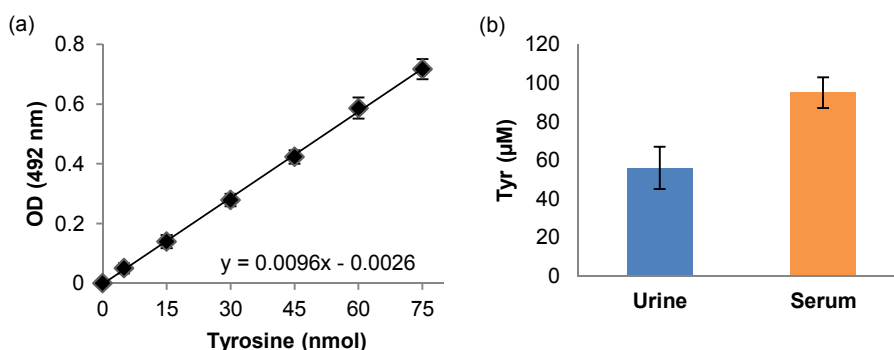


Figure: a) Tyrosine Standard Curve. b) Measurement of Tyrosine concentration in human urine & serum (135 μl , each). Both samples were deproteinized using 10 kDa Spin Column (Cat # 1997) & spiked with known amount of Tyrosine (30 nmol). Assays were performed following the kit protocol.

IX. Related Products:

Phenylalanine Fluorometric Assay Kit (K572)

Aspartate Colorimetric/Fluorometric Assay Kit (K552)

Branched Chain Amino Acid (Leu/Ile/Val) Colorimetric Assay Kit (K564)

L-Amino Acid Quantitation Colorimetric/Fluorometric Kit (K639)

Glutamate Colorimetric Assay Kit (K629)

Alanine Colorimetric/Fluorometric Assay Kit (K652)

Hydroxyproline Colorimetric Assay Kit (K555)

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