Tyrosine Assay kit

ARG83370 Tyrosine Assay kit is an assay kit for Tyrosine in Serum, plasma, saliva, urine, Cell culture supernatants, cell lysate and tissue lysates.

Catalog number: ARG83370

Package: 100 assay

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INTRODUCTION

L-tyrosine is an optically active form of tyrosine having L-configuration. It has a role as an EC 1.3.1.43 (arogenate dehydrogenase) inhibitor, a nutraceutical, a micronutrient and a fundamental metabolite. It is an erythrose 4-phosphate/phosphoenolpyruvate family amino acid, a proteinogenic amino acid, a tyrosine and a L-alpha-amino acid. It is functionally related to a L-tyrosinal. It is a conjugate base of a L-tyrosinium. It is a conjugate acid of a L-tyrosinate(1-). It is an enantiomer of a D-tyrosine. It is a tautomer of a L-tyrosine zwitterion.

PRINCIPLE OF THE ASSAY

ARG83370 Tyrosine Assay Kit measures Tyrosine content within biological samples. 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. The intensity of the color is measured at a wavelength of 490 nm. The concentration of Tyrosine in the sample is then determined by comparing the O.D. of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Standard and 10X Enzyme at -20°C.

Store 10X Assay Buffer at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Standard (100.0 mM)	100 µL	-20°C
10X Assay Buffer	25 ml	4°C
10X Enzyme	500 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 490 nm
- Flat bottomed 96-well black microplate and tube.
- 1X PBS and deionized water
- Pipettes and pipette tips

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Upon received, store Standard and 10X Enzyme at -20°C. Store 10X Assay Buffer at 4°C. Use the kit before expiration date. and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Saliva- Collect saliva using a collection device (e.g. Salivette), centrifuge 10,000 x g for 2 min at 4°C. Collect saliva and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles. The collection device should not have protein binding or filtering features.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at-80°C. Perform dilutions in PBS.

REAGENT PREPARATION

- 1X Assay Buffer: Dilute the 10X Assay Buffer with deionized water to yield 1X Assay Buffer. Store at RT.
- Reaction Mix: Dilute the 10X Enzyme at 1:10, HRP in 1X Assay Buffer. For 20 assays, mix 100 µL 10X Enzyme to 900 µL 1X Assay Buffer for total volume of 1 mL. Store the Reaction Mix at 4°C for 1 day.

- **Standards**: Add 5 µl of 100 mM stock standard into 495 µl 1X Assay Buffer to generate a standard with 1000 µM of Tyrosine. Dilute the standards with PBS serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard	Tyrosine (µM)	Volume of Assay Buffer (µL)	Volume of Tyrosine (µL)
S1	1000	495	5 (100 mM stock)
S2	500	250	250(S1)
S3	250	250	250(S2)
S4	125	250	250(S3)
S5	62.5	250	250(S4)
S6	31.3	250	250(S5)
S7	15.6	250	250(S6)
S8	0	250	0

ASSAY PROCEDURE

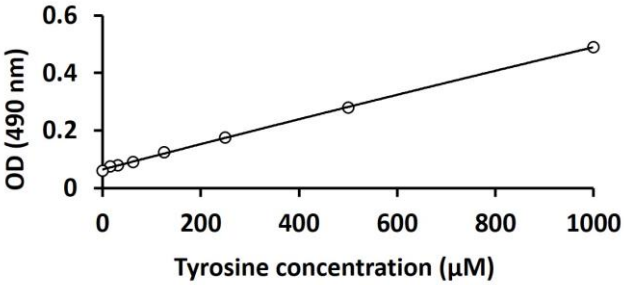
Standards and samples should be assayed in at least duplicates.

1. Add **50 µl** of **standard** and **sample** to each wells.
2. Add **50 µL** of **Reaction Mix** to **standard** each wells.
3. Mix well and Incubate for 10 min at RT.

   Note: This assay is continuous (not terminated), therefore may be measured at multiple time points to follow the reaction kinetics.
4. Read O.D. with a microplate reader at **490 nm** immediately.

EXAMPLE OF TYPICAL STANDARD CURVE

The following table shows the OD readings of a run of this assay kit with serial diluted standards



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Tyrosine ranged from 15.6-1000 µM. The mean MDD was 10 µM