

Tyrosine (Tyr) Assay Kit

Catalog No.: abx295232

Size: 96 tests

Detection Range: 0.06 mmol/L – 2 mmol/L

Sensitivity: 0.06 mmol/L

Storage: Store all components at -20°C in the dark.

Application: For the detection and quantification of Tyrosine (Tyr) concentration in serum, plasma, and tissue homogenates.

Principle of the Assay

Abbexa's Tyrosine (Tyr) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Tyrosine (Tyr) concentration. Tyrosinase catalyzes the oxidation of Tyrosine (Tyr) to dopachrome. The generated dopachrome reacts with the chromogenic agent 3-methyl-2-benzothiazolinone hydrazone (MBTH) to form a purple-red complex, which exhibits a maximum absorbance at 490 nm. The intensity of the color is proportional to the Tyrosine (Tyr) concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Assay Buffer: 20 ml
3. Enzyme Reagent: 2 vials
4. Standard: 2 vials
5. Chromogenic Reagent: 4 vials
6. Plate sealer: 2

Materials required but not provided

1. Microplate reader (490 nm)
2. Double-distilled water
3. DMSO
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Centrifuge and centrifuge tubes
7. Sonicator
8. Mechanical homogenizer
9. Vortex mixer
10. Incubator

Instructions for Use

Version: 1.0.1

Revision date: 8-Oct-26



Protocol

A. Preparation of samples and reagents

1. Reagents

- **Enzyme Reagent Working Solution:** Reconstitute 1 vial of Enzyme Reagent with 300 µl of double-distilled water. Mix thoroughly until fully dissolved. Unused Working Substrate Solution can be stored at -20°C for up to 14 days in the dark.
- **Chromogenic Reagent Working Solution:** Reconstitute 1 vial of Chromogenic Reagent with 280 µl of DMSO. Mix thoroughly and sonicate until fully dissolved. Unused Chromogenic Reagent Working Solution can be stored at -20°C for up to 3 days in the dark.
- **Activated Assay Solution:** Prepare enough Activated Assay Solution for the wells to be tested. Mix Chromogenic Reagent Working Solution, Enzyme Reagent Working Solution and Assay Buffer in a ratio of 4 : 5 : 141. For example, prepare 450 µl of Activated Assay Solution by mixing 12 µl of Chromogenic Reagent Working Solution, 15 µl of Enzyme Reagent Working Solution and 423 µl of Assay Buffer. Activated Assay Solution should be freshly prepared and used within 2 hours in the dark.
- **2 mmol/L Standard:** Reconstitute 1 vial of Standard with 8.3 ml of double-distilled water. Sonicate for 10–20 min until completely dissolved. Unused 2 mmol/L Standard can be stored at -20°C for up to 10 days in the dark. If precipitates are observed after freezing, sonicate until fully dissolved before use.
- **Standards:** Label 7 tubes with 0.2 mmol/L, 0.4 mmol/L, 0.6 mmol/L, 0.8 mmol/L, 1.2 mmol/L, 1.6 mmol/L, and 2 mmol/L. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (mmol/L)	0.2	0.4	0.6	0.8	1.2	1.6	2
2 mmol/L Standard (µl)	20	40	60	80	120	160	200
Double-distilled water (µl)	180	160	140	120	80	40	0

For the blank, use pure double-distilled water. The volume of each standard dilution will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

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The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and plasma:** Serum and plasma samples can be tested directly.
- **Tissue homogenates:** Carefully weigh at least 20 mg of tissue. Wash the tissue with cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add 180 µl of double-distilled water. Homogenize manually or use a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant for detection. Keep on ice and assay immediately.

Note: To calculate Tyrosine (Tyr) concentration in tissue homogenates using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (abx097193)

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with double-distilled water, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Mouse Kidney Tissue Homogenate	1
10% Mouse Liver Tissue Homogenate	1
10% Corn Tissue Homogenate	1
10% Potato Tissue Homogenate	1
Rat Serum	1
Human Serum	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the performance of this kit. It is recommended to use mechanical lysis methods only for tissue homogenates.

B. Assay Procedure

1. Mark positions on the 96-well microplate for each standard, blank, and sample. *Each sample requires a corresponding control. It is strongly recommended to test all wells in duplicate.*
2. Add 10 µl of each standard dilution to the standard wells.
3. Add 10 µl of double-distilled water to the blank wells.
4. Add 10 µl of sample to the sample wells.
5. Add 150 µl of Activated Assay Solution to all wells.

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- Mix the well contents by tapping the plate, or shaking with a microplate shaker, for approximately 5 seconds.
- Cover with a plate sealer, then incubate at 37°C for 30 minutes in the dark.
- Unseal the plate, then measure the OD of each well with a microplate reader at 490 nm.

C. Calculation of Results

Plot the standard curve, using the mean OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$.

Based on this curve, the concentration of Tyrosine (Tyr) in each sample well can be derived using the following formulae:

1. Liquid samples:

$$\text{Tyr (mmol/L)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}} - b)}{a} \times F$$

2. Tissue samples:

$$\text{Tyr (mmol/g protein)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}} - b)}{a \times C_{\text{Protein}}} \times F$$

where:

$\text{OD}_{\text{Sample}}$	OD value of the sample.
OD_{Blank}	OD value of the blank
C_{Protein}	Concentration of protein in sample (g/L)
a	Gradient of the standard curve ($y = ax + b$)
b	Y-intercept of the standard curve ($y = ax + b$)
F	Dilution factor of the sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.