

Instructions for Use

Version: 1.0.1

Revision date: 8-Oct-26



Tyrosinase Assay Kit

Catalog No.: abx295233

Size: 96 tests

Detection Range: 0.16 U/L – 15.57 U/L

Sensitivity: 0.16 U/L

Storage: Store all components at 4°C. Store the Substrate and Chromogenic Reagent in the dark.

Application: For the detection and quantification of Tyrosinase activity in serum, plasma, and tissue homogenates.

Principle of the Assay

Abbexa's Tyrosinase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Tyrosinase activity. Tyrosinase catalyzes the oxidation of dopamine to dopaquinone, which subsequently reacts with MBTH to form a pink-colored complex with an absorbance maximum at 505 nm. The intensity of the color is proportional to the Tyrosinase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Substrate: 4 vials
3. Assay Buffer: 4 × 45 ml
4. Chromogenic Reagent: 4 vials
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (505 nm)
2. DMSO
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Centrifuge and centrifuge tubes
6. Mechanical homogenizer
7. Vortex mixer
8. Incubator

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **Substrate Working Solution:** Reconstitute 1 vial of Substrate with 7.6 ml of Assay Buffer. Mix thoroughly until fully dissolved. Prepare the Substrate Working Solution immediately before use and use within 8 hours in the dark.
- **Chromogenic Reagent Working Solution:** Reconstitute 1 vial of Chromogenic Reagent with 0.14 ml of DMSO. Mix thoroughly until fully dissolved. Then add 3.36 mL of Assay Buffer and mix well. Prepare the Chromogenic Reagent Working Solution immediately before use and use within 8 hours in the dark.
- **Activated Assay Solution:** Prepare enough Activated Assay Solution for the wells to be tested. Mix Chromogenic Reagent Working Solution with Substrate Working Solution at a ratio of 1 : 4. For example, prepare 1250 µl of Activated Assay Solution by mixing 250 µl of Chromogenic Reagent Working Solution with 1000 µl of Substrate Working Solution. Prepare the Activated Assay Solution immediately before use and use within 8 hours in the dark.

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.

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 Assay Buffer. 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 Tyrosinase activity 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 Assay Buffer, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

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Sample Type	Dilution Factor
10% Mouse Kidney Tissue Homogenate	1
10% Mouse Liver Tissue Homogenate	1
10% Potato Tissue Homogenate	5 – 20
10% Sweet Potato Tissue Homogenate	2 – 10
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

- Mark positions on the 96-well microplate for each sample and control. *Each sample requires a corresponding control. It is strongly recommended to test all wells in duplicate.*
- Add 20 µl of sample to the sample wells.
- Add 20 µl of Assay Buffer to the corresponding control wells.
- Add 250 µl of Activated Assay Solution to all wells.
- Mix the well contents by tapping the plate, or shaking with a microplate shaker for at least 5 seconds.
- Measure the OD of each well with a microplate reader at 505 nm and record the values as A₁.
- Cover with a plate sealer, then incubate at 37°C for 20 minutes.
- Unseal the plate, then measure the OD of each well with a microplate reader at 505 nm and record the values as A₂.

C. Calculation of Results

For each sample, calculate the difference in absorbance between the two measurements: $\Delta OD = A_2 - A_1$.

1. Serum, plasma, and saliva samples:

$$\begin{aligned}\text{Tyrosinase activity (U/L)} &= \frac{(\Delta OD_{\text{Sample}} - \Delta OD_{\text{Control}}) \times V_{\text{Total}}}{\epsilon \times d \times T \times V_{\text{Sample}}} \times F \times 10^6 \\ &= (\Delta OD_{\text{Sample}} - \Delta OD_{\text{Control}}) \times 31.14 \times F\end{aligned}$$

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2. Tissue samples:

$$\begin{aligned}\text{Tyrosinase activity (U/g protein)} &= \frac{(\Delta\text{OD}_{\text{Sample}} - \Delta\text{OD}_{\text{Control}}) \times V_{\text{Total}}}{\epsilon \times d \times T \times V_{\text{Sample}} \times C_{\text{Protein}}} \times F \times 10^6 \\ &= \frac{(\Delta\text{OD}_{\text{Sample}} - \Delta\text{OD}_{\text{Control}})}{C_{\text{Protein}}} \times 31.14 \times F\end{aligned}$$

where:

$\Delta\text{OD}_{\text{Sample}}$	OD value of the sample.
$\Delta\text{OD}_{\text{Control}}$	OD value of the corresponding control
V_{Sample}	Volume of sample added to the microplate (20 μl)
V_{Total}	Total volume in the microplate (270 μl)
C_{Protein}	Concentration of protein in sample (g/L)
ϵ	Molar extinction coefficient of the chromogenic product ($2.89 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$)
d	Optical path length of the microplate well (0.75 cm)
T	Time for the reaction (20 minutes)
10^6	Conversion factor ($1 \text{ mol} = 1 \times 10^6 \mu\text{mol}$)
F	Dilution factor of the sample

Technical Support

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