Uricase (UOX) Assay Kit

Catalog No.: abx298960

Size: 96 tests

Detection Range: 0.06 U/L – 4 U/L

Sensitivity: 0.06 U/L

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

Application: For the detection and quantification of Uricase (UOX) activity in tissue homogenates.

Principle of the Assay

Abbexa's Uricase (UOX) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Uricase (UOX) activity. Uricase specifically catalyzes the oxidation of the substrate, which has an absorbance maximum at 293 nm. The reduction in absorbance at 293 nm is proportional to the Uricase (UOX) activity, which can then be calculated.

Kit components

1. 96-well UV microplate
2. Substrate: 8 vials
3. Assay Buffer: 2 × 35 ml
4. Matrix Solution: 12 ml
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (293 nm)
2. PBS (0.01 M, pH 7.4)
3. Pipette and pipette tips
4. Centrifuge and centrifuge tubes
5. Ultrasonic bath
6. Mechanical homogenizer
7. Vortex mixer
8. Incubator

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Substrate Stock Solution:** Reconstitute 1 vial of Substrate with 1 ml of Matrix Solution. Mix thoroughly using an ultrasonic bath until fully dissolved. Unused Substrate Stock Solution can be stored at -20°C for up to 3 days.
- **Substrate Working Solution:** Prepare enough Substrate Working Solution for the wells to be tested. Mix Substrate Stock Solution with Assay Buffer at a ratio of 1 : 29. For example, prepare 150 µl of Substrate Working Solution by mixing 5 µl of Substrate Stock Solution with 145 µl of Assay Buffer. Prepare fresh and use within 8 hours.
- **Assay Working Solution:** Prepare enough Assay Working Solution for the wells to be tested. Mix Substrate Working Solution and Assay Buffer at a ratio of 1 : 4. For example, prepare 160 µl of Assay Working Solution by mixing 32 µl of Substrate Working Solution with 128 µl of Assay Buffer. Prepare fresh and use within 8 hours.

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.

- **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.

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):

Sample Type	Dilution Factor
10% Mouse liver tissue homogenate	1 – 3
10% Porcine liver tissue homogenate	1 – 3
10% Spinach tissue homogenate	1

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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 blank, sample, and control. *Each sample requires a corresponding control. It is strongly recommended to test all wells in duplicate*.
2. Add 20 µl of Assay Buffer to the blank wells.
3. Add 20 µl of sample to the sample wells.
4. Add 20 µl of sample to the corresponding control wells.
5. Add 160 µl of Assay Working Solution to the blank and sample wells.
6. Add 160 µl of Assay Buffer to the control wells.
7. Mix thoroughly by tapping the plate, or shaking with a microplate shaker, for approximately 5 seconds.
8. Measure the OD of each well with a microplate reader at 293 nm and record the value as A_1.
9. Cover with a plate sealer, then incubate at 37°C for 20 minutes in the dark.
10. Unseal the plate, then measure the OD of each well with a microplate reader at 293 nm and record the value as A_2.

C. Calculation of Results

Calculate the difference in absorbance between the two measurements: $\Delta OD = A_1 - A_2$.

1. Tissue samples:

One unit of Uricase (UOX) activity is defined as the amount of enzyme that catalyzes the consumption of 1 µmol of uric acid per minute in 1 kg of tissue under the assay conditions at 37°C.

$$\text{UOX activity (U/Kg wet weight)} = \frac{\left(\Delta OD_{Sample} - \Delta OD_{Control} - \Delta OD_{Blank}\right) \times V_{Total} \times V_{Buffer}}{\varepsilon \times d \times T \times V_{Sample} \times W} \times F \times 10^6$$

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where:

ΔOD_{Sample}	$A_1 - A_2$ value of the sample.
$\Delta OD_{Control}$	$A_1 - A_2$value of the corresponding control
ΔOD_{Blank}	$A_1 - A_2$ value of the blank
V_{Total}	Total Volume in the microplate (0.18 ml)
V_{Sample}	Volume of sample added to the microplate (0.02 ml)
V_{Buffer}	Volume of Assay Buffer used during sample preparation (ml)
10^6	Conversion factor, where 1 mol/L = 10^6 µmol/L
ε	Molar extinction coefficient of uric acid (12,500 $L \cdot mol^{-1} \cdot cm^{-1}$)
d	Optical path length (0.6 cm)
T	Time for the reaction (20 minutes)
W	Wet weight of the tissue sample (g)
F	Dilution factor of the sample

Technical Support

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

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