Xanthine Oxidase (XOD) Assay Kit

Catalog No.: abx295042

Size: 96 tests

Detection Range: 0.01 U/L – 1.2 U/L

Sensitivity: 0.01 U/L

Storage: Store all components at -20°C. Store the Probe Solution and the Substrate in the dark.

Application: For detection and quantification of Xanthine Oxidase activity in serum, plasma, and tissue homogenates.

Introduction

Abbexa's Xanthine Oxidase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Xanthine Oxidase activity. Xanthine Oxidase oxidises hypoxanthine to produce xanthine and a super oxide anion. This super oxygen anion is converted into hydrogen peroxide in the system. The hydrogen peroxide then acts as a peroxidase and oxidises the probe solution to form a fluorescent substance. The fluorescence value can then be measured and this value is proportional to the Xanthine Oxidase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 60 ml
3. Probe Solution: 0.3 ml
4. H_2O_2 Standard Solution (2 mmol/L): 1.5 ml
5. Enzyme Reagent: 1 vial
6. Substrate: 4 ml
7. Plate sealer: 2

Materials required but not provided

1. Fluorescence Microplate reader (Excitation/ Emission= 535 nm/ 587 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Dounce homogenizer
8. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

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. Unused samples can be stored at -80°C for up to 1 month.
- **Tissue Homogenates:** Carefully weigh 0.02 g - 0.1 g of tissue and wash with cold PBS (0.01 M, pH 7.4). Add into 0.18 ml - 0.9 ml Buffer Solution (ratio 1:9). Homogenize manually, using a mechanical homogenizer, in an ice water bath. Collect the tissue homogenate, and centrifuge at 10,000 × g for 10 minutes. Carefully remove the supernatant and assay immediately, or aliquot and store at -80°C for up to 1 month.

  Note: To calculate Xanthine Oxidase activity in tissue homogenates using the formula 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 Buffer Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Mouse serum	1
Dog serum	3 – 5
Rat Plasma	1
Horse serum	1
Human plasma	1
10 % Rat kidney tissue homogenate	5 – 10
10 % Mouse heart tissue homogenate	5 – 10
10 % Rat lung tissue homogenate	5 - 10

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 kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Enzyme Working Solution:** Add 300 µl of Buffer Solution into one vial of Enzyme Reagent and mix thoroughly to dissolve to prepare Enzyme Working Solution. Unused Enzyme Working Solution can be aliquot and stored at -20°C for up to 30 days.
- **Sample Working Solution:** Per sample/ control sample add 46 µl of Substrate, 2 µl of Probe Solution, and 2 µl of Enzyme Working Solution to prepare 50 µl of Sample Working Solution. Prepare immediately before assaying and take care to avoid exposure to light.
- **Control Working Solution:** Per sample/ control sample add 46 µl of Buffer Solution, 2 µl of Probe Solution, and 2 µl of Enzyme Working Solution to prepare 50 µl of Sample Working Solution. Prepare immediately before assaying and take care to avoid exposure to light.
- **H_2O_2 Standard Solution (20 µmol/L):** Add 5 µl of H_2O_2 Standard Solution (2 mmol/L) to 495 µl of double-distilled water and mix thoroughly to prepare H_2O_2 Standard Solution (20 µmol/L). Prepare immediately before assaying.
- **Standards:** Label 7 tubes with 12 µmol/L, 10 µmol/L, 8 µmol/L, 6 µmol/L, 4 µmol/L, 2 µmol/L, and 1 µmol/L. Add 120 µl, 100 µl, 80 µl, 60 µl, 40 µl, 20 µl, and 10 µl of Standard (20 µmol/L) to the 12 µmol/L, 10 µmol/L, 8 µmol/L, 6 µmol/L, 4 µmol/L, 2 µmol/L, and 1 µmol/L tubes respectively, followed by 80 µl, 100 µl, 120 µl, 140 µl, 160 µl, 180 µl, and 190 µl of double-distilled water, to prepare Standard Dilutions with concentrations 12 µmol/L, 10 µmol/L, 8 µmol/L, 6 µmol/L, 4 µmol/L, 2 µmol/L, and 1 µmol/L. These volumes are summarized in the following table:

Standard Dilution (20 µmol/L)	**12**	**10**	**8**	**6**	**4**	**2**	**1**
20 µmol/L Standard (µl)	120	100	80	60	40	20	10
Double-distilled water (µl)	80	100	120	140	160	180	190

For the blank, or 0 µmol/L standard, use pure double-distilled water. The volume of each standard will be 200 µl.

Note:

- Reaction timing during the assay should be accurately monitored.
- The sample size of each batch is recommended to be no more than 20 to avoid effects on the recorded fluorescence values.

B. Assay Procedure

1. Record the positions of the wells required for each standard, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 50 µl of sample to each sample well, and 50 µl of the same sample to its corresponding control well.
3. Add 50 µl of each standard dilution to the corresponding standard wells.
4. Add 50 µl of Sample Working Solution to each standard and sample well
5. Add 50 µl of Control Working Solution to each control well.

6. Mix fully for 5 seconds using the microplate reader and leave to stand at room temperature for 2 minutes.
7. Measure the fluorescence intensity of each well with a microplate reader at the excitation wavelength of 535 nm and emission wavelength of 587 nm. Record this as F_1. Leave to stand at room temperature in the dark for 10 minutes.
8. Measure the fluorescence intensity of the wells at the excitation wavelength of 535 nm and emission wavelength of 587 nm. Record this as F_2.

$$F_{\text{Sample}} = F_{2\ (\text{Sample})} - F_{1\ (\text{Sample})}$$

$$F_{\text{Control}} = F_{2\ (\text{Control})} - F_{1\ (\text{Control})}$$

C. Calculation of Results

Plot the standard curve, using the absoluted F_2 values of the standard dilutions (adjusted for the blank/ 0 µmol/L standard) 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 activity of Xanthine Oxidase in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

One unit of Xanthine Oxidase activity is defined as the amount required for 1 L of serum or plasma to produce 1 µmol of H_2O_2 per minute at 25°C.

$$\text{Xanthine Oxidase Activity (U/L)} = f \times \frac{(F_{\text{Sample}} - F_{\text{Control}} - b)}{a \times t}$$

2. Tissue samples:

Xanthine Oxidase activity in tissue samples can be calculated according to total protein concentration. This can be determined separately (abx097193)

Total Protein

One unit of Xanthine Oxidase activity is defined as the amount required for 1 g of tissue protein to produce 1 µmol of H_2O_2 per minute at 25°C.

$$\text{Xanthine Oxidase Activity (U/g protein)} = f \times \frac{(F_{\text{Sample}} - F_{\text{Control}} - b)}{a \times t \times C_{\text{Protein}}}$$

where:

F_{Sample}	$F_{2\ (Sample)} - F_{1\ (Sample)}$
$F_{Control}$	$F_{2\ (Control)} - F_{1\ (Control)}$
$C_{Protein}$	Concentration of protein in sample (g protein/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time of the enzymatic reaction (10 mins)
f	The dilution factor of sample

Technical Support

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