New

LIFE SCIENCE

Xanthine Oxidase (XO) Activity Assay Kit

XO (Xanthine Oxidase) Activity Assay Kit

1kit [X0091]

This kit is designed to measure xanthine oxidase (XO) activity in biological samples. Xanthine oxidase is an essential enzyme involved in purine metabolism, converting xanthine and hypoxanthine into uric acid. It is widely used in the research of various oxidative stress-related diseases, such as gout and hyperuricemia. This kit measures the hydrogen peroxide produced by the action of xanthine oxidase, allowing for the calculation of xanthine oxidase activity based on the concentration of hydrogen peroxide.

Advantages

- Sensitively measures XO activity from 0 – 200 U/L
- Quick procedure with results within 2 hours
- Suitable for various biological samples

Kit Components

• 10 x XO Assay Buffer	1vial
• XO Standard	1vial
• XO Substrate Solution	1vial
• XO Enzyme	1vial
• Xanthine Solution	1vial

Each vial is sufficient for 96 tests.

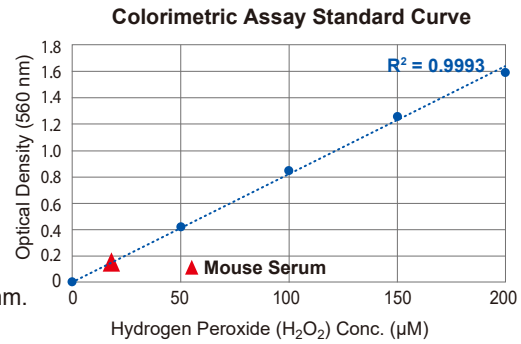
Example: XO activity test of mouse serum using X0091

‡ XO activity up to 200 U/L can be tested via the colorimetric assay.

1. **Prepare 1 x XO Assay Buffer**
   Mix 20 mL of 10 x XO Assay Buffer with 180 mL of distilled water.
2. **Prepare standard curve**
   2.1. Mix 1 µL of XO Standard (H_2O_2) with 102 µL of 1 x XO Assay Buffer to make a 10 mM H_2O_2 solution.
   2.2. Prepare the H_2O_2 standard solutions shown in the chart on the right table.
3. **Prepare standards and samples**
   Aliquot 50 µL of standards and samples to separate wells of a 96 well plate.
4. **Prepare reaction mixture**
   The volumes below represent the amount of each reagent needed per reaction per well. Mix an appropriate amount of each reagent for the number of wells to be assayed.
   - Xanthine Solution : 2 µL
   - XO Substrate : 1 µL
   - XO Enzyme : 1 µL
   - 1 x XO Assay Buffer : 46 µL
5. **Start reaction**
   Add 50 µL of reaction mixture (prepared in step 4) to each well and mix well.
6. **Measure Optical Density (OD)**
   6.1. As quickly as you can, measure OD1 at a wavelength between 550 - 580 nm.
   6.2. Incubate at 25 °C for 5 - 20 minutes and measure OD2 using the same conditions as above.
7. **Calculate activity**
   7.1. Plot standards to obtain a standard curve and calculate the slope.
   7.2. This slope, along with the OD change of the sample ΔOD_{Sample} = (OD2 – OD1) and blank ΔOD_{Blank} = (OD2 – OD1) can be used to calculate the sample XO activity based on the following formula:

$$\text{XO Activity (U/L)} = \frac{\Delta OD_{Sample} - \Delta OD_{Blank}}{\text{Slope} \times \textit{Time}} \times \text{Dilution ratio}$$

No.	10 mM H_2O_2	1 x XO Assay Buffer	Final Conc. (µM)
1	4 µL	196 µL	200
2	3 µL	197 µL	150
3	2 µL	198 µL	100
4	1 µL	199 µL	50
5	0 µL	200 µL	0



[Experimental Data]
Slope = 0.008196, *Time* = 20, "$\Delta OD_{Sample} - \Delta OD_{Blank}$" = 0.1484, Dilution ratio = 10
Based on the above data, the XO activity contained in mouse serum is 9.05 U/L. (Experiment condition : room temperature, pH 7.5)
‡ Prolong the reaction time if the value of OD is too weak to calculate the activity.

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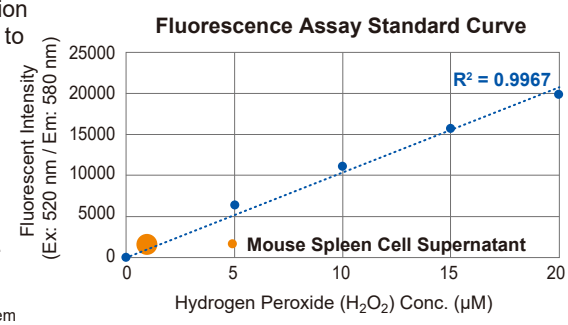
Example: XO activity test of mouse spleen cell supernatant using X0091

‡ XO activity from 0 - 20 U/L should be tested by fluorescence assay.

1. **Prepare 1 x XO Assay Buffer**
   Mix 20 mL of 10 x XO Assay Buffer with 180 mL of distilled water.
2. **Plot standard curve**
   2.1. Mix 1 µL of XO Standard (H_2O_2) with 102 µL of 1 x XO Assay Buffer to make a 10 mM H_2O_2solution.
   2.2. Mix 10 µL of 10 mM H_2O_2 solution with 90 µL of 1 x XO Assay Buffer to make a 1 mM H_2O_2 solution.
   2.3. Prepare the H_2O_2 standard solutions shown in the chart on the right table.
3. **Prepare standards and samples**
   Aliquot 50 µL of standards and samples to separate wells of a 96 well plate.
4. **Prepare reaction mixture**
   The volumes below represent the amount of each reagent needed per reaction per well. Mix an appropriate amount of each reagent for the number of wells to be assayed.
   - Xanthine Solution : 2 µL
   - XO Substrate : 0.1 µL
   - XO Enzyme : 1 µL
   - 1 x XO Assay Buffer : 47 µL
5. **Start reaction**
   Add 50 µL of reaction mixture (prepared in step 4) to each well and mix well.
6. **Measure Fluorescent intensity (F)**
   6.1. As quickly as you can, measure F1 using an λ_{ex} of 520 - 550 nm and λ_{em} of 585 - 595 nm.
   6.2. Incubate at 25 °C for 10 - 20 minutes and measure F2 using the same conditions as above.
7. **Calculate activity**
   7.1. Plot standards to obtain a standard curve and calculate the slope.
   7.2. This slope, along with the sample fluorescence intensity change ΔF_{Sample} = (F2 – F1) and blank fluorescence intensity change ΔF_{Blank} = (F2 – F1) can be used to calculate the sample XO activity based on the following formula:

$$\text{XO Activity (U/L)} = \frac{\Delta F_{Sample} - \Delta F_{Blank}}{\text{Slope} \times \textit{Time}} \times \text{Dilution ratio}$$

No.	1 mM H_2O_2	1 x XO Assay Buffer	Final Conc. (µM)
1	4 µL	196 µL	20
2	3 µL	197 µL	15
3	2 µL	198 µL	10
4	1 µL	199 µL	5
5	0 µL	200 µL	0



[Experimental Data]
Slope = 1038.22, *Time* = 20, "$\Delta F_{Sample} - \Delta F_{Blank}$" = 1494, Dilution ratio = 20
Based on the above data, the XO activity contained in the supernatant of mouse spleen cell is 1.44 U/L.
(Experiment condition : room temperature, pH 7.5)
‡ Prolong the reaction time if the value of fluorescent intensity is too weak to calculate the activity.

Ordering and Customer Service

TCI AMERICA
Tel : 800-423-8616 / 503-283-1681
Fax : 888-520-1075 / 503-283-1987
E-mail : Sales-US@TCIchemicals.com

TCI EUROPE N.V.
Tel : +32 (0)3 735 07 00
Fax : +32 (0)3 735 07 01
E-mail : Sales-EU@TCIchemicals.com

TCI Deutschland GmbH
Tel : +49 (0)6196 64053-00
Fax : +49 (0)6196 64053-01
E-mail : Sales-DE@TCIchemicals.com

Tokyo Chemical Industry UK Ltd.
Tel : +44 (0)1865 78 45 60
E-mail : Sales-UK@TCIchemicals.com

梯希爱(上海)化成工业发展有限公司
Tel : 800-988-0390 / 021-67121386
Fax : 021-6712-1385
E-mail : Sales-CN@TCIchemicals.com

Tokyo Chemical Industry (India) Pvt. Ltd.
Tel : 1800 425 7889 / 044-2262 0909
E-mail : Sales-IN@TCIchemicals.com

TOKYO CHEMICAL INDUSTRY CO., LTD.
Tel : +81 (0)3-5640-8878
E-mail : globalbusiness@TCIchemicals.com